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(54) Title: ISOPRENOID PRODUCTION

(57) Abstract: The invention provides methods and materials related to the production of isoprenoids. Specifically, the invention provides isolated nucleic acids, substantially pure polypeptides, host cells, and methods and materials for producing various isoprenoid compounds.

ISOPRENOID PRODUCTION

BACKGROUND

1. Technical Field

The invention relates to methods and materials involved in the production of isoprenoids.

2. Background Information

Isoprenoids are compounds that have at least one five-carbon isoprenoid unit. Examples of isoprenoid compounds include, without limitation, carotenoids, isoprenes, sterols, terpenes, and ubiquinones. Various enzymatic pathways in plants, animals, and microorganisms result in the synthesis of isoprenoid compounds. Typically, isopentenyl diphosphate (IPP), dimethylallyl diphosphate (DMAPP), or combinations thereof are polymerized to form isoprenoid compounds.

Two pathways can be used to produce IPP. The first pathway, known as the mevalonate-dependent pathway, produces IPP from 3-hydroxymethyl-3-methylglutaryl Coenzyme A (HMGCoA) in a series of reactions. The second pathway, known as the mevalonate-independent pathway, produces IPP from 1-deoxyxylulose-5-phosphate (DXP) in a series of reactions. One of those reactions involves the use of DXP synthase (DXS) to catalyze the condensation of pyruvate and glyceraldehyde-3-phosphate to form DXP.

Once made, IPP can be used to make various isoprenoid compounds. Specifically, enzymes known as polyprenyl diphosphate synthases catalyze polymerization reactions that combine IPP and DMAPP to form compounds known as polyprenyl diphosphates. For example, decaprenyl diphosphate synthase (DDS) catalyzes the consecutive condensation of IPP with allylic diphosphates to produce decaprenyl diphosphate. Decaprenyl diphosphate is a polyprenyl diphosphate that can be used to form the side chain of a ubiquinone known as CoQ(10). Other polyprenyl diphosphate synthases include, without limitation, farnesyl-, geranyl-, and octapreneyl diphosphate synthases.

SUMMARY

The invention relates to methods and materials involved in the production of isoprenoid compounds. Specifically, the invention provides nucleic acid molecules, polypeptides, host cells, and methods that can be used to produce isoprenoid compounds. Isoprenoid compounds are both biologically and commercially important. For example, the nutritional industry uses isoprenoid compounds as nutritional supplements, while the perfume industry uses isoprenoid compounds as fragrances. The nucleic acid molecules described herein can be used to engineer host cells having the ability to produce particular isoprenoid compounds. The polypeptides described herein can be used in cell-free systems to make particular isoprenoid compounds. The host cells described herein can be used in culture systems to produce large quantities of particular isoprenoid compounds.

In general, the invention features an isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:1 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (3626, 100), point B has coordinates (3626, 65), point C has coordinates (50, 65), and point D has coordinates (12, 100). The point B can have coordinates (3626, 85). The point C can have coordinates (100, 65). The point C can have coordinates (50, 85). The point D can have coordinates (15, 100). The nucleic acid sequence can encode a polypeptide. The polypeptide can have DXS activity. The nucleic acid sequence can be as set forth in SEQ ID NO:1.

In one embodiment, the invention features an isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:2 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1926, 100), point B has coordinates (1926, 65), point C has coordinates (50, 65), and point D has coordinates (12, 100). The nucleic acid sequence can encode a polypeptide. The polypeptide can have DXS activity.

In another embodiment, the invention features an isolated nucleic acid containing a nucleic acid sequence, wherein the nucleic acid sequence encodes a polypeptide containing an amino acid sequence, wherein the amino acid sequence has a length and a

percent identity to the sequence set forth in SEQ ID NO:3 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (641, 100), point B has coordinates (641, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100). The polypeptide can have DXS activity.

Another embodiment of the invention features an isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:37 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1990, 100), point B has coordinates (1990, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100). The point B can have coordinates (1990, 85). The point C can have coordinates (100, 55). The point C can have coordinates (50, 85). The point D can have coordinates (20, 100). The nucleic acid sequence can encode a polypeptide. The polypeptide can have DDS activity. The nucleic acid sequence can be as set forth in SEQ ID NO:37.

Another embodiment of the invention features an isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:38 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1002, 100), point B has coordinates (1002, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100). The nucleic acid sequence can encode a polypeptide. The polypeptide can have DDS activity.

Another embodiment of the invention features an isolated nucleic acid containing a nucleic acid sequence, wherein the nucleic acid sequence encodes a polypeptide containing an amino acid sequence, wherein the amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:39 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (333, 100), point B has coordinates (333, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100). The polypeptide can have DDS activity.

Another embodiment of the invention features an isolated nucleic acid containing

a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:40 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1833, 100), point B has coordinates (1833, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100). The point B can have coordinates (1833, 85). The point C can have coordinates (100, 65). The point C can have coordinates (50, 85). The point D can have coordinates (20, 100). The nucleic acid sequence can encode a polypeptide. The polypeptide can have DDS activity. The nucleic acid sequence can be as set forth in SEQ ID NO:40.

Another embodiment of the invention features an isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:41 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1014, 100), point B has coordinates (1014, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100). The nucleic acid sequence can encode a polypeptide. The polypeptide can have DDS activity.

Another embodiment of the invention features an isolated nucleic acid containing a nucleic acid sequence, wherein the nucleic acid sequence encodes a polypeptide containing an amino acid sequence, wherein the amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:42 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (337, 100), point B has coordinates (337, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100). The polypeptide can have DDS activity.

Another embodiment of the invention features an isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:95 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (2017, 100), point B has coordinates (2017, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100). The point B can have coordinates (2017, 85). The point C can have coordinates (100, 65). The point C can have coordinates (50,

85). The point D can have coordinates (20, 100). The nucleic acid sequence can encode a polypeptide. The polypeptide can have DXR activity. The nucleic acid sequence can be as set forth in SEQ ID NO:95.

Another embodiment of the invention features an isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:96 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1161, 100), point B has coordinates (1161, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100). The nucleic acid sequence can encode a polypeptide. The polypeptide can have DXR activity.

Another embodiment of the invention features an isolated nucleic acid containing a nucleic acid sequence, wherein the nucleic acid sequence encodes a polypeptide containing an amino acid sequence, wherein the amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:97 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (386, 100), point B has coordinates (386, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100). The polypeptide can have DXR activity.

Another embodiment of the invention features an isolated nucleic acid containing a nucleic acid sequence of at least 12 nucleotides, wherein the isolated nucleic acid hybridizes under hybridization conditions to the sense or antisense strand of a nucleic acid molecule, the sequence of the nucleic acid molecule being the sequence set forth in SEQ ID NO: 1, 2, 37, 38, 40, 41, 95, or 96. The nucleic acid sequence can be at least 50 nucleotides (e.g., at least 100, 200, 300, 400, 500, or more). The nucleic acid sequence can encode a polypeptide. The polypeptide can have DXS, DDS, or DXR activity.

In another aspect, the invention features a substantially pure polypeptide containing an amino acid sequence, wherein the amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:3 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (641, 100), point B has coordinates (641, 65), point C has coordinates (25, 65), and point D has coordinates (5,

100). The polypeptide can have DXS activity.

In another embodiment, the invention features a substantially pure polypeptide containing an amino acid sequence, wherein the amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:39 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (333, 100), point B has coordinates (333, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100). The polypeptide can have DDS activity.

Another embodiment of the invention features a substantially pure polypeptide containing an amino acid sequence, wherein the amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:42 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (337, 100), point B has coordinates (337, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100). The polypeptide can have DDS activity.

Another embodiment of the invention features a substantially pure polypeptide containing an amino acid sequence, wherein the amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:97 over the length, wherein the point defined by the length and the percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (386, 100), point B has coordinates (386, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100). The polypeptide can have DXR activity.

Another aspect of the invention features a host cell containing an isolated nucleic acid of claim 1, 9, 12, 14, 22, 25, 27, 35, 38, 40, 48, 51, or 53. The host cell can be prokaryotic. The host cell can be a *Rhodobacter*, *Sphingomonas*, or *Escherichia* cell. The host cell can contain an exogenous nucleic acid that encodes a polypeptide having DDS, DXS, ODS, SDS, DXR, 4-diphosphocytidyl-2C-methyl-D-erythritol synthase, 4-diphosphocytidyl-2C-methyl-D-erythritol kinase, or chorismate lyase activity. The host cell can contain an exogenous nucleic acid containing an UbiC sequence or LytB sequence. The host cell can contain an exogenous nucleic acid containing an UbiC sequence and LytB sequence. The host cell can contain a non-functional crtE sequence,

ppsR sequence, or ccoN sequence. The host cell can contain a non-functional crtE sequence, ppsR sequence, and ccoN sequence.

Another embodiment of the invention features a host cell containing an exogenous nucleic acid and a non-functional crtE sequence, ppsR sequence, or ccoN sequence, wherein the exogenous nucleic acid is within a crtE, ppsR, or ccoN locus of the host cell.

Another embodiment of the invention features a host cell containing a genomic deletion, wherein the deletion comprises at least a portion of a crtE sequence, ppsR sequence, or ccoN sequence, and wherein the host cell comprises a non-functional crtE sequence, ppsR sequence, or ccoN sequence.

Another aspect of the invention features a method for increasing production of CoQ(10) in a cell having endogenous DDS activity. The method includes inserting a nucleic acid molecule containing a nucleic acid sequence that encodes a polypeptide having DDS activity into the cell such that production of CoQ(10) is increased. The nucleic acid molecule can contain an isolated nucleic acid of claim 14, 22, 25, 27, 35, 38, or 53. The production of CoQ(10) can be increased at least about 5 percent as compared to a control cell lacking the inserted nucleic acid molecule. The cell can be a *Rhodobacter* or *Sphingomonas* cell. The cell can be a membraneous bacterium or highly membraneous bacterium. The method can also include inserting a second nucleic acid molecule containing a nucleotide sequence that encodes a polypeptide having DXS activity into the cell. The second nucleic acid molecule can contain an isolated nucleic acid of claim 1, 9, or 12.

In another embodiment, the invention features a method for increasing production of CoQ(10) in a cell having endogenous DDS activity. The method includes inserting a nucleic acid molecule containing a nucleic acid sequence that encodes a polypeptide having DXS activity into the cell such that production of CoQ(10) is increased. The production of CoQ(10) can be increased at least about 5 percent as compared to a control cell lacking the inserted nucleic acid molecule. The cell can be a *Rhodobacter* or *Sphingomonas* cell. The nucleic acid molecule can contain an isolated nucleic acid of claim 1, 9, or 12. The cell can be a membraneous bacterium or highly membraneous bacterium. The method can also include inserting a second nucleic acid molecule containing a nucleotide sequence that encodes a polypeptide having DDS activity into the

cell. The second nucleic acid molecule can contain an isolated nucleic acid of claim 14, 22, 25, 27, 35, 38, or 53.

Another embodiment of the invention features a method for increasing production of CoQ(10) in a membraneous bacterium. The method includes inserting a nucleic acid molecule containing a nucleic acid sequence that encodes a polypeptide having DDS activity into the bacterium such that production of CoQ(10) is increased.

Another embodiment of the invention features a method for increasing production of CoQ(10) in a highly membraneous bacterium. The method includes inserting a nucleic acid molecule containing a nucleic acid sequence that encodes a polypeptide having DDS activity into the highly membraneous bacterium such that production of CoQ(10) is increased.

Another embodiment of the invention features a method for making an isoprenoid. The method includes culturing a cell under conditions wherein the cell produces the isoprenoid, wherein the cell contains at least one exogenous nucleic acid that encodes at least one polypeptide, wherein the cell produces more of the isoprenoid than a comparable cell lacking the at least one exogenous nucleic acid. The cell can be a *Rhodobacter* or *Sphingomonas* cell. The isoprenoid can be CoQ(10). The at least one polypeptide can have DDS, DXS, ODS, SDS, DXR, 4-diphosphocytidyl-2C-methyl-D-erythritol synthase, 4-diphosphocytidyl-2C-methyl-D-erythritol kinase, or chorismate lyase activity. The at least one polypeptide can be a UbiC polypeptide or a LytB polypeptide. The cell can contain a non-functional crtE sequence, ppsR sequence, or ccoN sequence. The cell can contain a non-functional crtE sequence, ppsR sequence, and ccoN sequence. The cell can contain a genomic deletion, wherein the deletion contains at least a portion of a crtE sequence, ppsR sequence, or ccoN sequence, and wherein the cell contains a non-functional crtE sequence, ppsR sequence, or ccoN sequence.

Another embodiment of the invention features a method for making an isoprenoid. The method includes culturing a genetically modified cell under conditions wherein the cell produces the isoprenoid. The isoprenoid can be CoQ(10). The cell can contain an exogenous nucleic acid. The cell can contain a genomic deletion.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this

invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

DESCRIPTION OF DRAWINGS

Figure 1 is a diagram of a pathway for producing CoQ(10).

Figure 2 is a listing of a nucleic acid sequence that encodes a *Sphingomonas trueperi* (ATCC 12417) polypeptide having DXS activity (SEQ ID NO:1). The start codon is the ATG at nucleotide number 182, and the stop codon is the TAA at nucleotide number 2107. The probable ribosome binding site is at nucleotide numbers 175-178. This sequence contains an open reading frame as well as 5' and 3' untranslated sequences.

Figure 3 is a listing of a nucleic acid sequence that encodes a *Sphingomonas trueperi* (ATCC 12417) polypeptide having DXS activity (SEQ ID NO:2). This sequence corresponds to the open reading frame.

Figure 4 is a listing of an amino acid sequence of a *Sphingomonas trueperi* (ATCC 12417) polypeptide having DXS activity (SEQ ID NO:3).

Figure 5 is a sequence pile-up of 14 nucleic acid sequences that encode polypeptides having DXS activity. STdxsdna represents the nucleic acid sequence set forth in SEQ ID NO:2; CRdxsdna represents a nucleic acid sequence from *Chlamydomonas reinhardtii* (GenBank accession number AJ007559; SEQ ID NO:4); CJdxsdna represents a nucleic acid sequence from *Campylobacter jejuni* (GenBank accession number AL139074; SEQ ID NO:5); PAdxsdna represents a nucleic acid sequence from *Pseudomonas aeruginosa* (GenBank accession number AE004821; SEQ ID NO:6); LEdxsdna represents a nucleic acid sequence from *Lycopersicon esculentum* (GenBank accession number AF143812; SEQ ID NO:7); MTdxsdna represents a nucleic

acid sequence from *Mycobacterium tuberculosis* (GenBank accession number Z96072; ; SEQ ID NO:8); RSdxs1dna represents a nucleic acid sequence from a *Rhodobacter sphaeroides* dxs1 gene (SEQ ID NO:9); RSdxs2dna represents a nucleic acid sequence from a *Rhodobacter sphaeroides* dxs2 gene (SEQ ID NO:10); SPCCdxsdna represents a nucleic acid sequence from *Synechococcus* PCC6301 (GenBank accession number Y18874; SEQ ID NO:11); ECdxsdna represents a nucleic acid sequence from *Escherichia coli* (GenBank accession number AF035440; SEQ ID NO:12); NMdxsdna represents a nucleic acid sequence from *Neisseria meningitidis* (GenBank accession number AL162753; SEQ ID NO:13); HIdxsdna represents a nucleic acid sequence from *Haemophilus influenza* (GenBank accession number U32822; SEQ ID NO:14); SSdxsdna represents a nucleic acid sequence from *Streptomyces sp.* CL190 (GenBank accession number AB026631; SEQ ID NO:16); and HPdxsdna represents a nucleic acid sequence from *Helicobacter pylori* 26695 (GenBank accession number AE000552; SEQ ID NO:17).

Figure 6 is a sequence pile-up of 21 amino acid sequences of polypeptides having DXS activity. STdxsp represents an amino acid sequence set forth in SEQ ID NO:3; AAdxsp represents an amino acid sequence from *Aquifex aeolicus* (GenBank accession number O67036; SEQ ID NO:18); BSdxsp represents an amino acid sequence from *Bacillus subtilis* (GenBank accession number P54523; SEQ ID NO:19); CRdxsp represents an amino acid sequence from *Chlamydomonas reinhardtii* (GenBank accession number CAA07554; SEQ ID NO:20); CJdxsp represents an amino acid sequence from *Campylobacter jejuni* (GenBank accession number CAB72788; SEQ ID NO:21); PAdxsp represents an amino acid sequence from *Pseudomonas aeruginosa* (GenBank accession number AAG07431; SEQ ID NO:15); LEdxsp represents an amino acid sequence from *Lycopersicon esculentum* (GenBank accession number AAD38941; SEQ ID NO:22); MLdxsp represents an amino acid sequence from *Mycobacterium leprae* (GenBank accession number Q50000; SEQ ID NO:23); MTdxsp represents an amino acid sequence from *Mycobacterium tuberculosis* (GenBank accession number CAB09493; SEQ ID NO:24); RCdxsp represents an amino acid sequence from *Rhodobacter capsulatus* (GenBank accession number P26242; SEQ ID NO:25); RSdxs1p represents an amino acid sequence encoded by a *Rhodobacter sphaeroides* dxs1 gene (SEQ ID NO:26);

RSdxs2p represents an amino acid sequence encoded by a *Rhodobacter sphaeroides* dxs2 gene (SEQ ID NO:27); SPCCdxsp represents an amino acid sequence from *Synechococcus* PCC6301 (GenBank accession number CAB60078; SEQ ID NO:28); SPdxsp represents an amino acid sequence from *Synechocystis* PCC6803 (GenBank accession number P73067; SEQ ID NO:29); TMdxsp represents an amino acid sequence from *Thermotoga maritima* (GenBank accession number Q9X291; SEQ ID NO:30); ECdxsp represents an amino acid sequence from *Escherichia coli* (GenBank accession number D64771; SEQ ID NO:31); NMdxsp represents an amino acid sequence from *Neisseria meningitidis* (GenBank accession number CAB83880; SEQ ID NO:32); HIdxsp represents an amino acid sequence from *Haemophilus influenza* (GenBank accession number B64172; SEQ ID NO:33); PFdxsp represents an amino acid sequence from *Plasmodium falciparum* (GenBank accession number AAD03740; SEQ ID NO:34); SSdxsp represents an amino acid sequence from *Streptomyces sp.* CL190 (GenBank accession number BAA85847; SEQ ID NO:35); and HPdxsp represents an amino acid sequence from *Helicobacter pylori* 26695 (GenBank accession number AAD07422; SEQ ID NO:36).

Figure 7 is a listing of a nucleic acid sequence that encodes a *Rhodobacter sphaeroides* (ATCC 17023) polypeptide having DDS activity (SEQ ID NO:37). The start codon is the ATG at nucleotide number 372, and the stop codon is the TGA at nucleotide number 1373. The probable ribosome binding site is at nucleotide numbers 363-366. This sequence contains an open reading frame as well as 5' and 3' untranslated sequences.

Figure 8 is a listing of a nucleic acid sequence that encodes a *Rhodobacter sphaeroides* (ATCC 17023) polypeptide having DDS activity (SEQ ID NO:38). This sequence corresponds to the open reading frame.

Figure 9 is a listing of an amino acid sequence of a *Rhodobacter sphaeroides* (ATCC 17023) polypeptide having DDS activity (SEQ ID NO:39).

Figure 10 is a listing of a nucleic acid sequence that encodes a *Sphingomonas trueperi* (ATCC 12417) polypeptide having DDS activity (SEQ ID NO:40). The start codon is the ATG at nucleotide number 605, and the stop codon is the TGA at nucleotide number 1618. The probable ribosome binding site is at nucleotide numbers 590-594.

This sequence contains an open reading frame as well as 5' and 3' untranslated sequences.

Figure 11 is a listing of a nucleic acid sequence that encodes a *Sphingomonas trueperi* (ATCC 12417) polypeptide having DDS activity (SEQ ID NO:41). This sequence corresponds to the open reading frame.

Figure 12 is a listing of an amino acid sequence of a *Sphingomonas trueperi* (ATCC 12417) polypeptide having DDS activity (SEQ ID NO:42). This sequence corresponds to the open reading frame.

Figure 13 is a sequence pile-up of five nucleic acid sequences that encode polypeptides having DDS activity. RSddsdna represents the nucleic acid sequence set forth in SEQ ID NO:38; STddsdna represents the nucleic acid sequence set forth in SEQ ID NO:41; SPddsdna represents a nucleic acid sequence from *Schizosaccharomyces pombe* (GenBank accession number D84311; SEQ ID NO:43); GSddsdna represents a nucleic acid sequence from *Gluconobacter suboxydans* (GenBank accession number AB006850; SEQ ID NO:44); and RCddsdna represents a nucleic acid sequence from *Rhodobacter capsulatus* (U.S. Patent No. 6,103,488; SEQ ID NO:45).

Figure 14 is a sequence pile-up of five amino acid sequences of polypeptides having DDS activity. RSddsp represents the amino acid sequence set forth in SEQ ID NO:39; STddsp represents the amino acid sequence set forth in SEQ ID NO:42; GSddsp represents an amino acid sequence from *Gluconobacter suboxydans* (GenBank accession number BAA32241; SEQ ID NO:46); SPddsp represents an amino acid sequence from *Schizosaccharomyces pombe* (GenBank accession number CAB66154; SEQ ID NO:47); and RCddsp represents an amino acid sequence from *Rhodobacter capsulatus* (U.S. Patent No. 6,103,488; SEQ ID NO:48).

Figure 15 is a sequence pile-up of three amino acid sequences of polypeptides having DXS activity. Hpdxsp represents the amino acid sequence set forth in SEQ ID NO:36; Ecdxsp represents the amino acid sequence set forth in SEQ ID NO:31; and Hidxsp represents the amino acid sequence set forth in SEQ ID NO:33.

Figure 16 is a sequence pile-up of four amino acid sequences of polypeptides having DDS, ODS (octaprenyl diphosphate synthase), or SDS (solanesyl diphosphate synthase) activity. Rcsdsp represents an amino acid sequence from *Rhodobacter*

capsulatus having SDS activity (SEQ ID NO:49); Rpodsp represents an amino acid sequence from *Rickettsia prowazeki* having ODS activity (SEQ ID NO:50); Gsddsp represents the amino acid sequence set forth in SEQ ID NO:46; and Ecodsp represents an amino acid sequence from *Escherichia coli ispB* having ODS activity (SEQ ID NO:51).

Figure 17 is a sequence pile-up of five amino acid sequences of polypeptides having DDS, ODS, or SDS activity. Rpodsp represents the amino acid sequence set forth in SEQ ID NO:50; Gsddsp represents the amino acid sequence set forth in SEQ ID NO:46; Ecodsp represents the amino acid sequence set forth in SEQ ID NO:51; Hiodsp represents an amino acid sequence from *Haemophilus influenze* having ODS activity (SEQ ID NO:52); and Rcsdsp represents the amino acid sequence set forth in SEQ ID NO:49.

Figure 18 is a diagram of a construct designated appUC18-SHDXS.

Figure 19 is a diagram of a construct designated appUC18-RSdds.

Figure 20 is a diagram of a construct designated appUC18-SHDDS.

Figure 21 is a mass chromatogram obtained from a MG1655 PUC18 specimen.

Figure 22 is a mass chromatogram obtained from a MG1655 PUC18-DDS specimen.

Figure 23 is a mass spectra obtained from a MG1655 PUC18 specimen.

Figure 24 is a mass spectra obtained from a MG1655 PUC18-DDS specimen.

Figure 25 is a mass spectra obtained from a MG1655 PUC18-DDS specimen.

Figure 26 is a graph plotting length and percent identity with points A, B, C, and D defining an area indicated by shading.

Figure 27 is a sequence pile-up of seven amino acid sequences of polypeptides having DXR activity. Bsdxrp represents an amino acid sequence from *Bacillus subtilis* (SEQ ID NO:98); Hmdxrp represents an amino acid sequence from *Haemophilus influenzae* (SEQ ID NO:99); Ecdxrp represents an amino acid sequence from *Escherishia coli* (SEQ ID NO:100); Zmdxrp represents an amino acid sequence from *Zymonas mobilis* (SEQ ID NO:101); Sldxrp represents an amino acid sequence from *Synechococcus leopoliensis* (SEQ ID NO:102); Ssdxrp represents an amino acid sequence from *Synechocystis sp.* PCC6803 (SEQ ID NO:103); and Mtdxrp represents an amino acid sequence from *Mycobacterium tuberculosis* (SEQ ID NO:104).

Figure 28 is a listing of a nucleic acid sequence that encodes a *Sphingomonas trueperi* polypeptide having DXR activity (SEQ ID NO:95). The start codon is the GTG at either nucleotide number 575 or 578, and the stop codon is the TGA at nucleotide number 1733. This sequence contains an open reading frame as well as 5' and 3' untranslated sequences.

Figure 29 is a listing of a nucleic acid sequence that encodes a *Sphingomonas trueperi* polypeptide having DXR activity (SEQ ID NO:96). This sequence corresponds to the open reading frame.

Figure 30 is a listing of an amino acid sequence of a *Sphingomonas trueperi* polypeptide having DXR activity (SEQ ID NO:97).

Figure 31 is a sequence pile-up of twelve nucleic acid sequences that encode polypeptides having DXR activity. Stdxrcds represents the nucleic acid sequence set forth in SEQ ID NO:96; Padxrd represents a nucleic acid sequence from *Pseudomonas aeruginosa* (SEQ ID NO:105); Zmdxrd represents a nucleic acid sequence from *Zygomonas mobilis* (SEQ ID NO:106); Sgdxrd represents a nucleic acid sequence from *Streptomyces griseolosporeus* (SEQ ID NO:107); Nmdxrd represents a nucleic acid sequence from *Neisseria meningitidis* (SEQ ID NO:108); Ecdxrd represents a nucleic acid sequence from *Escherishia coli* (SEQ ID NO:109); Sldxrd represents a nucleic acid sequence from *Synechococcus leopoliensis* (SEQ ID NO:110); Mldxrd represents a nucleic acid sequence from *Mycobacterium leprae* (SEQ ID NO:111); Pmdxrd represents a nucleic acid sequence from *Pasteurella multocida* (SEQ ID NO:112); Atdxrd represents a nucleic acid sequence from *Arabidopsis thaliana* (SEQ ID NO:113); Cjdxrd represents a nucleic acid sequence from *Campylobacter jejuni* (SEQ ID NO:114); and Pfdxrd represents a nucleic acid sequence from *Plasmodium falciparum* (SEQ ID NO:115).

Figure 32 is a sequence pile-up of sixteen amino acid sequences of polypeptides having DXR activity. Stdxrp represents the amino acid sequence set forth in SEQ ID NO:97; Zmdxrp represents an amino acid sequence from *Zymononas mobilis* (SEQ ID NO:116); Padxrp represents an amino acid sequence from *Pseudomonas aeruginosa* (SEQ ID NO:117); Ecdxrp represents an amino acid sequence from *Escherishia coli* (SEQ ID NO:118); Nmdxrp represents an amino acid sequence from *Neisseria meningitidis* (SEQ ID NO:119); Hidxrp represents an amino acid sequence from

Haemophilus influenzae (SEQ ID NO:120); Ssdxrp represents an amino acid sequence from *Synechocystis sp.* PCC6803 (SEQ ID NO:121); Pmdxrp represents an amino acid sequence from *Pasteurella multocida* (SEQ ID NO:122); Sldxrp represents an amino acid sequence from *Synechococcus leopoliensis* (SEQ ID NO:123); Sgdxrp represents an amino acid sequence from *Streptomyces griseolosporeus* (SEQ ID NO:124); Bsdxrp represents an amino acid sequence from *Bacillus subtilis* (SEQ ID NO:125); Mldxrp represents an amino acid sequence from *Mycobacterium leprae* (SEQ ID NO:126); Mtdxrp represents an amino acid sequence from *Mycobacterium tuberculosis* (SEQ ID NO:127); Atdxrp represents an amino acid sequence from *Arabidopsis thaliana* (SEQ ID NO:128); Cjdxrp represents an amino acid sequence from *Campylobacter jejuni* (SEQ ID NO:130); and Pfdxrp represents an amino acid sequence from *Plasmodium falciparum* (SEQ ID NO:131).

DETAILED DESCRIPTION

The invention provides methods and materials related to the production of isoprenoids. Specifically, the invention provides isolated nucleic acids, substantially pure polypeptides, host cells, and methods and materials for producing various isoprenoid compounds. For the purpose of this invention, an isoprenoid compound is any compound containing a five-carbon isoprenoid unit. Examples of isoprenoid compounds include, without limitation, carotenoids, isoprenes, sterols, terpenes, and ubiquinones. Such isoprenoid compounds can be used in a wide range of applications. For example, isoprenoid compounds produced as described herein can be used in industrial, pharmaceutical, or cosmetic products.

In general terms, carotenoids are lipophilic pigments typically found in photosynthetic plants and bacteria. Examples of carotenoids include, without limitation, carotenes, xanthophylls, hydrocarbon carotenoids, hydroxy carotenoid derivatives, epoxy carotenoid derivatives, furanoxy carotenoid derivatives, and oxy carotenoid derivatives. Isoprenes are oily hydrocarbons that can be obtained by distilling caoutchouc or guttaipercha. Examples of isoprenes include, without limitation, rubber, vitamin A, and vitamin K. Sterols are steroid-based alcohols typically having a hydrocarbon side-chain of eight to ten carbon atoms at the 17-beta position and a hydroxyl group at the 3-beta

position. Examples of sterols include, without limitation, ergosterol, cholesterol, and stigmasterol. Terpenes are lipid species typically found in plants in great abundance. Examples of terpenes include, without limitation, dolichol, squalene, and limonene. Ubiquinones are 2,3-dimethoxy-5-methylbenzoquinone derivatives having a side chain containing at least one isoprenoid unit. Typically, ubiquinone is referred to as Coenzyme Q (CoQ). In addition, the number of isoprenoid units of a side chain of a particular ubiquinone is used to identify that particular ubiquinone. For example, a ubiquinone with six isoprenoid units is referred to as CoQ(6), while a ubiquinone with ten isoprenoid units is referred to as CoQ(10). It is noted that CoQ(10) also is referred to as ubidecarenone. Examples of ubiquinones include, without limitation, CoQ(6), CoQ(8), CoQ(10), and CoQ(12).

Isoprenoid compounds can be pyruvate-derived products. The term "pyruvate-derived product" as used herein refers to any compound that is synthesized from pyruvate within no more than 25 enzymatic steps. Thus, an isoprenoid compound is not a pyruvate-derived product if that isoprenoid compound is synthesized from pyruvate in more than 25 enzymatic steps. An enzymatic step is a single chemical reaction catalyzed by a polypeptide having enzymatic activity. The term "polypeptide having enzymatic activity" as used herein refers to any polypeptide that catalyzes a chemical reaction of other substances without itself being destroyed or altered upon completion of the reaction. Typically, a polypeptide having enzymatic activity catalyzes the formation of one or more products from one or more substrates. Such polypeptides can have any type of enzymatic activity including, without limitation, the enzymatic activity associated with an enzyme such as DXS, DDS, ODS, SDS, DXR (1-deoxy-D-xylulose 5-phosphate reductoisomerase), ispD (4-diphosphocytidyl-2C-methyl-D-erythritol synthase), and ispE (4-diphosphocytidyl-2C-methyl-D-erythritol kinase).

A polypeptide having a particular enzymatic activity can be a polypeptide that is either naturally-occurring or non-naturally-occurring. A naturally-occurring polypeptide is any polypeptide having an amino acid sequence as found in nature, including wild-type and polymorphic polypeptides. Such naturally-occurring polypeptides can be obtained from any species including, without limitation, animal (e.g., mammalian), plant, fungal, and bacterial species. A non-naturally-occurring polypeptide is any polypeptide having

an amino acid sequence that is not found in nature. Thus, a non-naturally-occurring polypeptide can be a mutated version of a naturally-occurring polypeptide, or an engineered polypeptide. For example, a non-naturally-occurring polypeptide having DDS activity can be a mutated version of a naturally-occurring polypeptide having DDS activity that retains at least some DDS activity. A polypeptide can be mutated by, for example, sequence additions, deletions, substitutions, or combinations thereof.

Examples of isoprenoid compounds that are pyruvate-derived products include, without limitation, CoQ(6), CoQ(7), CoQ(8), CoQ(9), CoQ(10), astaxanthin, canthaxanthin, lutein, zeaxanthin, beta-carotene, lycopene, capsanthin, bixin, norbixin, crocetin, zeta-carotene, vitamin E, giberellins, abscisic acid, ergosterol, geraniol, and latex.

As depicted in Figure 1, multiple polypeptide can be used to convert glucose CoQ(10). For example, polypeptides having DXS, DXR, LytB, and DDS activity can be used to convert glucose CoQ(10). Such polypeptides can be obtained and used to make CoQ(10) as described herein.

1. Nucleic acids

The term "nucleic acid" as used herein encompasses both RNA and DNA, including cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. The nucleic acid can be double-stranded or single-stranded. Where single-stranded, the nucleic acid can be the sense strand or the antisense strand. In addition, nucleic acid can be circular or linear.

The term "isolated" as used herein with reference to nucleic acid refers to a naturally-occurring nucleic acid that is not immediately contiguous with both of the sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally-occurring genome of the organism from which it is derived. For example, an isolated nucleic acid can be, without limitation, a recombinant DNA molecule of any length, provided one of the nucleic acid sequences normally found immediately flanking that recombinant DNA molecule in a naturally-occurring genome is removed or absent. Thus, an isolated nucleic acid includes, without limitation, a recombinant DNA that exists as a separate molecule (e.g., a cDNA or a genomic DNA

fragment produced by PCR or restriction endonuclease treatment) independent of other sequences as well as recombinant DNA that is incorporated into a vector, an autonomously replicating plasmid, a virus (e.g., a retrovirus, adenovirus, or herpes virus), or into the genomic DNA of a prokaryote or eukaryote. In addition, an isolated nucleic acid can include a recombinant DNA molecule that is part of a hybrid or fusion nucleic acid sequence.

The term "isolated" as used herein with reference to nucleic acid also includes any non-naturally-occurring nucleic acid since non-naturally-occurring nucleic acid sequences are not found in nature and do not have immediately contiguous sequences in a naturally-occurring genome. For example, non-naturally-occurring nucleic acid such as an engineered nucleic acid is considered to be isolated nucleic acid. Engineered nucleic acid can be made using common molecular cloning or chemical nucleic acid synthesis techniques. Isolated non-naturally-occurring nucleic acid can be independent of other sequences, or incorporated into a vector, an autonomously replicating plasmid, a virus (e.g., a retrovirus, adenovirus, or herpes virus), or the genomic DNA of a prokaryote or eukaryote. In addition, a non-naturally-occurring nucleic acid can include a nucleic acid molecule that is part of a hybrid or fusion nucleic acid sequence.

It will be apparent to those of skill in the art that a nucleic acid existing among hundreds to millions of other nucleic acid molecules within, for example, cDNA or genomic libraries, or gel slices containing a genomic DNA restriction digest is not to be considered an isolated nucleic acid.

The term "exogenous" as used herein with reference to nucleic acid and a particular cell refers to any nucleic acid that does not originate from that particular cell as found in nature. Thus, all non-naturally-occurring nucleic acid is considered to be exogenous to a cell once introduced into the cell. It is important to note that non-naturally-occurring nucleic acid can contain nucleic acid sequences or fragments of nucleic acid sequences that are found in nature provided the nucleic acid as a whole does not exist in nature. For example, a nucleic acid molecule containing a genomic DNA sequence within an expression vector is non-naturally-occurring nucleic acid, and thus is exogenous to a cell once introduced into the cell, since that nucleic acid molecule as a whole (genomic DNA plus vector DNA) does not exist in nature. Thus, any vector,

autonomously replicating plasmid, or virus (e.g., retrovirus, adenovirus, or herpes virus) that as a whole does not exist in nature is considered to be non-naturally-occurring nucleic acid. It follows that genomic DNA fragments produced by PCR or restriction endonuclease treatment as well as cDNAs are considered to be non-naturally-occurring nucleic acid since they exist as separate molecules not found in nature. It also follows that any nucleic acid containing a promoter sequence and polypeptide-encoding sequence (e.g., cDNA or genomic DNA) in an arrangement not found in nature is non-naturally-occurring nucleic acid.

Nucleic acid that is naturally-occurring can be exogenous to a particular cell. For example, an entire chromosome isolated from a cell of person X is an exogenous nucleic acid with respect to a cell of person Y once that chromosome is introduced into Y's cell.

The invention provides isolated nucleic acid that contains a nucleic acid sequence having (1) a length, and (2) a percent identity to an identified nucleic acid sequence over that length. The invention also provides isolated nucleic acid that contains a nucleic acid sequence encoding a polypeptide that contains an amino acid sequence having (1) a length, and (2) a percent identity to an identified amino acid sequence over that length. Typically, the identified nucleic acid or amino acid sequence is a sequence referenced by a particular sequence identification number, and the nucleic acid or amino acid sequence being compared to the identified sequence is referred to as the target sequence. For example, an identified sequence can be the sequence set forth in SEQ ID NO: 1.

A length and percent identity over that length for any nucleic acid or amino acid sequence is determined as follows. First, a nucleic acid or amino acid sequence is compared to the identified nucleic acid or amino acid sequence using the BLAST 2 Sequences (Bl2seq) program from the stand-alone version of BLASTZ containing BLASTN version 2.0.14 and BLASTP version 2.0.14. This stand-alone version of BLASTZ can be obtained from the University of Wisconsin library as well as at www.fr.com or www.ncbi.nlm.nih.gov. Instructions explaining how to use the Bl2seq program can be found in the readme file accompanying BLASTZ. Bl2seq performs a comparison between two sequences using either the BLASTN or BLASTP algorithm. BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. To compare two nucleic acid sequences, the options are set as

follows: -i is set to a file containing the first nucleic acid sequence to be compared (e.g., C:\seq1.txt); -j is set to a file containing the second nucleic acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastn; -o is set to any desired file name (e.g., C:\output.txt); -q is set to –1; -r is set to 2; and all other options are left at their default setting. For example, the following command can be used to generate an output file containing a comparison between two sequences: C:\Bl2seq –i c:\seq1.txt –j c:\seq2.txt –p blastn –o c:\output.txt –q –1 –r 2. To compare two amino acid sequences, the options of Bl2seq are set as follows: -i is set to a file containing the first amino acid sequence to be compared (e.g., C:\seq1.txt); -j is set to a file containing the second amino acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastp; -o is set to any desired file name (e.g., C:\output.txt); and all other options are left at their default setting. For example, the following command can be used to generate an output file containing a comparison between two amino acid sequences: C:\Bl2seq –i c:\seq1.txt –j c:\seq2.txt –p blastp –o c:\output.txt. If the target sequence shares homology with any portion of the identified sequence, then the designated output file will present those regions of homology as aligned sequences. If the target sequence does not share homology with any portion of the identified sequence, then the designated output file will not present aligned sequences. Once aligned, a length is determined by counting the number of consecutive nucleotides or amino acid residues from the target sequence presented in alignment with sequence from the identified sequence starting with any matched position and ending with any other matched position. A matched position is any position where an identical nucleotide or amino acid residue is presented in both the target and identified sequence. Gaps presented in the target sequence are not counted since gaps are not nucleotides or amino acid residues. Likewise, gaps presented in the identified sequence are not counted since target sequence nucleotides or amino acid residues are counted, not nucleotides or amino acid residues from the identified sequence.

The percent identity over a determined length is determined by counting the number of matched positions over that length and dividing that number by the length followed by multiplying the resulting value by 100. For example, if (1) a 1000 nucleotide target sequence is compared to the sequence set forth in SEQ ID NO:1, (2) the Bl2seq program presents 200 nucleotides from the target sequence aligned with a region of the

sequence set forth in SEQ ID NO: 1 where the first and last nucleotides of that 200 nucleotide region are matches, and (3) the number of matches over those 200 aligned nucleotides is 180, then the 1000 nucleotide target sequence contains a length of 200 and a percent identity over that length of 90 (i.e. 180 ÷ 200 * 100 = 90).

It will be appreciated that a single nucleic acid or amino acid target sequence that aligns with an identified sequence can have many different lengths with each length having its own percent identity. For example, a target sequence containing a 20 nucleotide region that aligns with an identified sequence as follows has many different lengths including those listed in Table 1.

```
                              1                   20
Target Sequence:              AGGTCGTGTACTGTCAGTCA
                              |  ||  |||  ||||  |||| |
Identified Sequence:          ACGTGGTGAACTGCCAGTGA
```

Table I.

Starting Position	Ending Position	Length	Matched Positions	Percent Identity
1	20	20	15	75.0
1	18	18	14	77.8
1	15	15	11	73.3
6	20	15	12	80.0
6	17	12	10	83.3
6	15	10	8	80.0
8	20	13	10	76.9
8	16	9	7	77.8

It is noted that the percent identity value is rounded to the nearest tenth. For example, 78.11, 78.12, 78.13, and 78.14 is rounded down to 78.1, while 78.15, 78.16, 78.17, 78.18, and 78.19 is rounded up to 78.2. It is also noted that the length value will always be an integer.

The invention provides an isolated nucleic acid containing a nucleic acid sequence that has at least one length and percent identity over that length as determined above such that the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26. In addition, the invention provides an isolated nucleic

acid containing a nucleic acid sequence that encodes a polypeptide containing an amino acid sequence that has at least one length and percent identity over that length as determined above such that the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26. The point defined by a length and percent identity over that length is that point on the X/Y coordinate of Figure 26 where the X axis is the length and the Y axis is the percent identity. Thus, the point defined by a nucleic acid sequence with a length of 200 and a percent identity of 90 has coordinates (200, 90). For the purpose of this invention, any point that falls on point A, B, C, or D is considered within the area defined by points A, B, C, and D of Figure 26. Likewise, any point that falls on a line that defines the area defined by points A, B, C, and D is considered within the area defined by points A, B, C, and D of Figure 26.

It will be appreciated that the term "the area defined by points A, B, C, and D of Figure 26" as used herein refers to that area defined by the lines that connect point A with point B, point B with point C, point C with point D, and point D with point A. Points A, B, C, and D can define an area having any shape defined by four points (e.g., square, rectangle, or rhombus). In addition, two or more points can have the same coordinates. For example, points B and C can have identical coordinates. In this case, the area defined by points A, B, C, and D of Figure 26 is triangular. If three points have identical coordinates, then the area defined by points A, B, C, and D of Figure 26 is a line. In this case, any point that falls on that line would be considered within the area defined by points A, B, C, and D of Figure 26. If all four points have identical coordinates, then the area defined by points A, B, C, and D of Figure 26 is a point. In all cases, simple algebraic equations can be used to determine whether a point is within the area defined by points A, B, C, and D of Figure 26.

It is noted that Figure 26 is a graphical representation presenting possible positions of points A, B, C, and D. The shaded area illustrated in Figure 26 represents one possible example, while the arrows indicate that other positions for points A, B, C, and D are possible. In fact, points A, B, C, and D can have any X coordinate and any Y coordinate. For example, point A can have an X coordinate equal to the number of nucleotides or amino acid residues in an identified sequence, and a Y coordinate of 100. Point B can have an X coordinate equal to the number of nucleotides or amino acid

residues in an identified sequence, and a Y coordinate less than or equal to 100 (e.g., 50, 55, 65, 70, 75, 80, 85, 90, 95, and 99). Point C can have an X coordinate equal to a percent (e.g., 1, 2, 5, 10, 15, or more percent) of the number of nucleotides or amino acid residues in an identified sequence, and a Y coordinate less than or equal to 100 (e.g., 50, 55, 65, 70, 75, 80, 85, 90, 95, and 99). Point D can have an X coordinate equal to the length of a typical PCR primer (e.g., 12, 13, 14, 15, 16, 17, or more) or antigenic polypeptide (e.g., 5, 6, 7, 8, 9, 10, 11, 12, or more), and a Y coordinate less than or equal to 100 (e.g., 50, 55, 65, 70, 75, 80, 85, 90, 95, and 99).

An isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:1 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 3626, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 3626, and a Y coordinate greater than or equal to 65; where point C has an X coordinate greater than or equal to 50, and a Y coordinate greater than or equal to 65; and where point D has an X coordinate greater than or equal to 12, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 3626, 3600, 3500, 3000, 2500, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 3626, 3600, 3500, 3000, 2500, or less; and the Y coordinate for point B can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (3626, 100), point B can be (3626, 95), point C can be (1900, 95), and point D can be (1900, 100).

An isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:2 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 1926, and a Y coordinate less than or equal to 100; where

point B has an X coordinate less than or equal to 1926, and a Y coordinate greater than or equal to 65; where point C has an X coordinate greater than or equal to 50, and a Y coordinate greater than or equal to 65; and where point D has an X coordinate greater than or equal to 12, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 1926, 1900, 1850, 1800, 1750, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 1926, 1900, 1850, 1800, 1750, or less; and the Y coordinate for point B can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (1926, 100), point B can be (1926, 95), point C can be (1000, 95), and point D can be (1000, 100).

An isolated nucleic acid containing a nucleic acid sequence that encodes a polypeptide containing an amino acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:3 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 641, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 641, and a Y coordinate greater than or equal to 50; where point C has an X coordinate greater than or equal to 25, and a Y coordinate greater than or equal to 50; and where point D has an X coordinate greater than or equal to 5, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 641, 635, 630, 625, 620, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 641, 635, 630, 625, 620, or less; and the Y coordinate for point B can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (641, 100), point B can be (641, 95), point C can be

(400, 95), and point D can be (400, 100).

An isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:37 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 1990, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 1990, and a Y coordinate greater than or equal to 65; where point C has an X coordinate greater than or equal to 50, and a Y coordinate greater than or equal to 65; and where point D has an X coordinate greater than or equal to 12, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 1990, 1950, 1900, 1850, 1800, 1750, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 1990, 1950, 1900, 1850, 1800, 1750, or less; and the Y coordinate for point B can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (1990, 100), point B can be (1990, 95), point C can be (1000, 95), and point D can be (1000, 100).

An isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:38 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 1002, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 1002, and a Y coordinate greater than or equal to 65; where point C has an X coordinate greater than or equal to 50, and a Y coordinate greater than or equal to 65; and where point D has an X coordinate greater than or equal to 12, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 1002, 950, 900, 850, 800, 750, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 1002, 950, 900, 850, 800, 750, or less; and the Y coordinate for point B can be 65, 70,

75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (1002, 100), point B can be (1002, 95), point C can be (500, 95), and point D can be (500, 100).

An isolated nucleic acid containing a nucleic acid sequence that encodes a polypeptide containing an amino acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:39 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 333, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 333, and a Y coordinate greater than or equal to 50; where point C has an X coordinate greater than or equal to 25, and a Y coordinate greater than or equal to 50; and where point D has an X coordinate greater than or equal to 5, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 333, 330, 325, 320, 315, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 333, 330, 325, 320, 315, or less; and the Y coordinate for point B can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (333, 100), point B can be (333, 95), point C can be (150, 95), and point D can be (150, 100).

An isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:40 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 1833, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 1833, and a Y coordinate greater than or

equal to 65; where point C has an X coordinate greater than or equal to 50, and a Y coordinate greater than or equal to 65; and where point D has an X coordinate greater than or equal to 12, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 1833, 1800, 1750, 1700, 1650, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 1833, 1800, 1750, 1700, 1650, or less; and the Y coordinate for point B can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (1833, 100), point B can be (1833, 95), point C can be (900, 95), and point D can be (900, 100).

An isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:41 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 1014, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 1014, and a Y coordinate greater than or equal to 65; where point C has an X coordinate greater than or equal to 50, and a Y coordinate greater than or equal to 65; and where point D has an X coordinate greater than or equal to 12, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 1014, 950, 900, 800, 700, 600, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 1014, 950, 900, 800, 700, 600, or less; and the Y coordinate for point B can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (1014, 100), point B can be (1014, 95), point C can be (500, 95), and point D can be (500, 100).

An isolated nucleic acid containing a nucleic acid sequence that encodes a

polypeptide containing an amino acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:42 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 337, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 337, and a Y coordinate greater than or equal to 50; where point C has an X coordinate greater than or equal to 25, and a Y coordinate greater than or equal to 50; and where point D has an X coordinate greater than or equal to 5, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 337, 335, 330, 325, 320, 315, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 337, 335, 330, 325, 320, 315, or less; and the Y coordinate for point B can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (337, 100), point B can be (337, 95), point C can be (150, 95), and point D can be (150, 100).

An isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:95 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 2017, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 2017, and a Y coordinate greater than or equal to 65; where point C has an X coordinate greater than or equal to 50, and a Y coordinate greater than or equal to 65; and where point D has an X coordinate greater than or equal to 12, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 2017, 2000, 1900, 1950, 1800, 1700, 1600, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 2017, 2000, 1900, 1950, 1800, 1700, 1600, or less; and the Y coordinate for point B can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C

can be 50, 60, 70, 80, 90, 100, 150, 200, 500, 1000, 1500, or more; and the Y coordinate for point C can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, 250, 500, 1000, 1500, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (2017, 100), point B can be (2017, 95), point C can be (1800, 95), and point D can be (1800, 100).

An isolated nucleic acid containing a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:96 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 1161, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 1161, and a Y coordinate greater than or equal to 65; where point C has an X coordinate greater than or equal to 50, and a Y coordinate greater than or equal to 65; and where point D has an X coordinate greater than or equal to 12, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 1161, 1050, 1000, 950, 900, 800, 700, 600, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 1161, 1050, 1000, 950, 900, 800, 700, 600, or less; and the Y coordinate for point B can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 50, 60, 70, 80, 90, 100, 150, 200, 250, 500, 1000, or more; and the Y coordinate for point C can be 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, 250, 500, 1000, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (1161, 100), point B can be (1161, 95), point C can be (1000, 95), and point D can be (1000, 100).

An isolated nucleic acid containing a nucleic acid sequence that encodes a polypeptide containing an amino acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:97 over that length is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 386, and a Y coordinate less than or equal to 100; where point B has an X

coordinate less than or equal to 386, and a Y coordinate greater than or equal to 50; where point C has an X coordinate greater than or equal to 25, and a Y coordinate greater than or equal to 50; and where point D has an X coordinate greater than or equal to 5, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 386, 380, 375, 370, 375, 360, 365, 350, 325, 300, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 386, 380, 375, 370, 375, 360, 365, 350, 325, 300, or less; and the Y coordinate for point B can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 350, or more; and the Y coordinate for point C can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, 200, 300, 350, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (386, 100), point B can be (386, 95), point C can be (350, 95), and point D can be (350, 100).

The invention also provides isolated nucleic acid that is at least about 12 bases in length (e.g., at least about 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 60, 100, 250, 500, 750, 1000, 1500, 2000, 3000, 4000, or 5000 bases in length) and hybridizes, under hybridization conditions, to the sense or antisense strand of a nucleic acid having the sequence set forth in SEQ ID NO:1, 2, 37, 38, 40, 41, 95, or 96. The hybridization conditions can be moderately or highly stringent hybridization conditions.

For the purpose of this invention, moderately stringent hybridization conditions mean the hybridization is performed at about 42°C in a hybridization solution containing 25 mM KPO_4 (pH 7.4), 5X SSC, 5X Denhart's solution, 50 μg/mL denatured, sonicated salmon sperm DNA, 50% formamide, 10% Dextran sulfate, and 1-15 ng/mL probe (about $5x10^7$ cpm/μg), while the washes are performed at about 50°C with a wash solution containing 2X SSC and 0.1% sodium dodecyl sulfate.

Highly stringent hybridization conditions mean the hybridization is performed at about 42°C in a hybridization solution containing 25 mM KPO_4 (pH 7.4), 5X SSC, 5X Denhart's solution, 50 μg/mL denatured, sonicated salmon sperm DNA, 50% formamide, 10% Dextran sulfate, and 1-15 ng/mL probe (about $5x10^7$ cpm/μg), while the washes are performed at about 65°C with a wash solution containing 0.2X SSC and 0.1% sodium

dodecyl sulfate.

Isolated nucleic acid within the scope of the invention can be obtained using any method including, without limitation, common molecular cloning and chemical nucleic acid synthesis techniques. For example, PCR can be used to obtain an isolated nucleic acid containing a nucleic acid sequence sharing similarity to the sequence set forth in SEQ ID NO:1, 2, 37, 38, 40, 41, 95, or 96. PCR refers to a procedure or technique in which target nucleic acid is amplified in a manner similar to that described in U.S. Patent No. 4,683,195, and subsequent modifications of the procedure described therein. Generally, sequence information from the ends of the region of interest or beyond are used to design oligonucleotide primers that are identical or similar in sequence to opposite strands of a potential template to be amplified. Using PCR, a nucleic acid sequence can be amplified from RNA or DNA. For example, a nucleic acid sequence can be isolated by PCR amplification from total cellular RNA, total genomic DNA, and cDNA as well as from bacteriophage sequences, plasmid sequences, viral sequences, and the like. When using RNA as a source of template, reverse transcriptase can be used to synthesize complimentary DNA strands.

An isolated nucleic acid within the scope of the invention also can be obtained by mutagenesis. For example, an isolated nucleic acid containing a sequence set forth in SEQ ID NO:1, 2, 37, 38, 40, 41, 95, or 96 can be mutated using common molecular cloning techniques (e.g., site-directed mutagenesis). Possible mutations include, without limitation, deletions, insertions, and substitutions, as well as combinations of deletions, insertions, and substitutions.

In addition, nucleic acid and amino acid databases (e.g., GenBank®) can be used to obtain an isolated nucleic acid within the scope of the invention. For example, any nucleic acid sequence having some homology to a sequence set forth in SEQ ID NO:1, 2, 37, 38, 40, 41, 95, or 96, or any amino acid sequence having some homology to a sequence set forth in SEQ ID NO:3, 39, 42, or 97 can be used as a query to search GenBank®.

Further, nucleic acid hybridization techniques can be used to obtain an isolated nucleic acid within the scope of the invention. Briefly, any nucleic acid having some homology to a sequence set forth in SEQ ID NO:1, 2, 37, 38, 40, 41, 95, or 96 can be

used as a probe to identify a similar nucleic acid by hybridization under conditions of moderate to high stringency. Once identified, the nucleic acid then can be purified, sequenced, and analyzed to determine whether it is within the scope of the invention as described herein.

Hybridization can be done by Southern or Northern analysis to identify a DNA or RNA sequence, respectively, that hybridizes to a probe. The probe can be labeled with a biotin, digoxygenin, an enzyme, or a radioisotope such as ^{32}P. The DNA or RNA to be analyzed can be electrophoretically separated on an agarose or polyacrylamide gel, transferred to nitrocellulose, nylon, or other suitable membrane, and hybridized with the probe using standard techniques well known in the art such as those described in sections 7.39-7.52 of Sambrook *et al.*, (1989) Molecular Cloning, second edition, Cold Spring harbor Laboratory, Plainview, NY. Typically, a probe is at least about 20 nucleotides in length. For example, a probe corresponding to a 20 nucleotide sequence set forth in SEQ ID NO:1, 2, 37, 38, 40, 41, 95, or 96 can be used to identify an identical or similar nucleic acid. In addition, probes longer or shorter than 20 nucleotides can be used.

The invention provides isolated nucleic acid that contains the entire nucleic acid sequence depicted in Figure 2, 3, 7, 8, 10, 11, 28, or 29. In addition, the invention provides isolated nucleic acid that contains a portion of the nucleic acid sequence depicted in Figure 2, 3, 7, 8, 10, 11, 28, or 29. For example, the invention provides isolated nucleic acid that contains a 15 nucleotide sequence identical to any 15 nucleotide sequence depicted in Figure 2, 3, 7, 8, 10, 11, 28, or 29 including, without limitation, the sequence starting at nucleotide number 1 and ending at nucleotide number 15, the sequence starting at nucleotide number 2 and ending at nucleotide number 16, the sequence starting at nucleotide number 3 and ending at nucleotide number 17, and so forth. It will be appreciated that the invention also provides isolated nucleic acid that contains a nucleotide sequence that is greater than 15 nucleotides (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more nucleotides) in length and identical to any portion of the sequence depicted in Figure 2, 3, 7, 8, 10, 11, 28, or 29. For example, the invention provides isolated nucleic acid that contains a 25 nucleotide sequence identical to any 25 nucleotide sequence depicted in Figure 2, 3, 7, 8, 10, 11, 28, or 29 including, without limitation, the sequence starting at nucleotide number 1 and ending at nucleotide

variations. For example, the STdxsdna sequence can contain one variation provided in Figure 5 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 5. It is noted that the full-length nucleic acid sequences depicted in Figure 5 can encode polypeptides having DXS activity. It also is noted that the nucleic acid sequence depicted in Figure 2 contains the nucleic acid sequence depicted in Figure 3.

Figure 13 depicts the nucleic acid sequence depicted in Figure 8 (designated RSddsdna) and the nucleic acid sequence depicted in Figure 11 (designated STddsdna) aligned with each other as well as aligned with three other nucleic acid sequences. Examples of variations of the RSddsdna sequence include, without limitation, any variation of the RSddsdna sequence provided in Figure 13. Examples of variations of the STddsdna sequence include, without limitation, any variation of the STddsdna sequence provided in Figure 13. Such variations are provided in Figure 13 in that a comparison of the nucleotide (or lack thereof) at a particular position of the RSddsdna sequence or the STddsdna sequence with the nucleotide (or lack thereof) at the same position of any of the other nucleic acid sequences depicted in Figure 13 provides a list of specific changes for the RSddsdna sequence and the STddsdna sequence. For example, the "a" at position 511 of the RSddsdna sequence or the "a" at position 756 of the STddsdna sequence can be substituted with an "t" as indicated in Figure 13. Again, it will be appreciated that the RSddsdna sequence as well as the STddsdna sequence can contain any number of variations as well as any combination of types of variations. For example, the RSddsdna sequence can contain one variation provided in Figure 13 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 13. Likewise, the STddsdna sequence can contain one variation provided in Figure 13 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 13. It is noted that the full-length nucleic acid sequences depicted in Figure 13 can encode polypeptides having DDS activity. It also is noted that the nucleic acid sequence depicted in Figure 7 contains the nucleic acid sequence depicted in Figure 8 and that the nucleic acid sequence depicted in Figure 10 contains the nucleic acid sequence depicted in Figure 11.

The nucleic acid sequence depicted in Figure 7 contains a nucleic acid sequence

that encodes a *R. sphaeroides* (ATCC 17023) polypeptide having DDS activity. Another variant of this nucleic acid sequence is the nucleic acid sequence of a clone isolated from *R. sphaeroides* (ATCC 35053). Briefly, a *R. sphaeroides* (ATCC 35053) clone was identified and found to contain a sequence identical to the nucleic acid sequence depicted in Figure 7 with the following three exceptions. The *R. sphaeroides* (ATCC 35053) clone has a "t" at position 885 rather than a "c", a "c" inserted after the "c" at position 1620, and a "c" inserted after the "c" at position 1733.

The nucleic acid depicted in Figure 8 also contains a nucleic acid sequence that encodes a *R. sphaeroides* (ATCC 17023) polypeptide having DDS activity. Another variant of this nucleic acid sequence is the nucleic acid sequence of a clone isolated from *R. sphaeroides* (ATCC 35053). Briefly, a *R. sphaeroides* (ATCC 35053) clone was identified and found to contain a sequence identical to the nucleic acid sequence depicted in Figure 8 with the following exception. The *R. sphaeroides* (ATCC 35053) clone has a "t" at position 514 rather than a "c".

Figure 31 depicts the nucleic acid sequence depicted in Figure 29 (designated Stdxrcds) aligned with eleven other nucleic acid sequences. Examples of variations of the Stdxrcds sequence include, without limitation, any variation of the Stdxrcds sequence provided in Figure 31. Such variations are provided in Figure 31 in that a comparison of the nucleotide (or lack thereof) at a particular position of the Stdxrcds sequence with the nucleotide (or lack thereof) at the same position of any of the other nucleic acid sequences depicted in Figure 31 provides a list of specific changes for the Stdxrcds sequence. Again, it will be appreciated that the Stdxrcds sequence can contain any number of variations as well as any combination of types of variations. For example, the Stdxrcds sequence can contain one variation provided in Figure 31 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 31. It is noted that the full-length nucleic acid sequences depicted in Figure 31 can encode polypeptides having DXR activity. It also is noted that the nucleic acid sequence depicted in Figure 29 contains the nucleic acid sequence depicted in Figure 28.

The invention also provides isolated nucleic acid that contains a variant of a portion of the nucleic acid sequence depicted in Figure 2, 3, 7, 8, 10, 11, 28, or 29 as described herein.

The invention provides isolated nucleic acid that contains a nucleic acid sequence that encodes the entire amino acid sequence depicted in Figure 4, 9, 12, or 30. In addition, the invention provides isolated nucleic acid that contains a nucleic acid sequence that encodes a portion of the amino acid sequence depicted in Figure 4, 9, 12, or 30. For example, the invention provides isolated nucleic acid that contains a nucleic acid sequence that encodes a 15 amino acid sequence identical to any 15 amino acid sequence depicted in Figure 4, 9, 12, or 30 including, without limitation, the sequence starting at amino acid residue number 1 and ending at amino acid residue number 15, the sequence starting at amino acid residue number 2 and ending at amino acid residue number 16, the sequence starting at amino acid residue number 3 and ending at amino acid residue number 17, and so forth. It will be appreciated that the invention also provides isolated nucleic acid that contains a nucleic acid sequence that encodes an amino acid sequence that is greater than 15 amino acid residues (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more amino acid residues) in length and identical to any portion of the sequence depicted in Figure 4, 9, 12, or 30. For example, the invention provides isolated nucleic acid that contains a nucleic acid sequence that encodes a 25 amino acid sequence identical to any 25 amino acid sequence depicted in Figure 4, 9, 12, or 30 including, without limitation, the sequence starting at amino acid residue number 1 and ending at amino acid residue number 25, the sequence starting at amino acid residue number 2 and ending at amino acid residue number 26, the sequence starting at amino acid residue number 3 and ending at amino acid residue number 27, and so forth. Additional examples include, without limitation, isolated nucleic acids that contain a nucleic acid sequence that encodes an amino acid sequence that is 50 or more amino acid residues (e.g., 100, 150, 200, 250, 300, 350, or more amino acid residues) in length and identical to any portion of the sequence depicted in Figure 4, 9, 12, or 30. Such isolated nucleic acids can include, without limitation, those isolated nucleic acids containing a nucleic acid sequence that encodes an amino acid sequence represented in a single line of sequence depicted in Figure 4, 9, 12, or 30 since each line of sequence depicted in these figures, with the exception of the last line, provides a 50 amino acid sequence.

In addition, the invention provides isolated nucleic acid that contains a nucleic acid sequence that encodes an amino acid sequence having a variation of the amino acid

sequence depicted in Figure 4, 9, 12, or 30. For example, the invention provides isolated nucleic acid containing a nucleic acid sequence encoding an amino acid sequence depicted in Figure 4, 9, 12, or 30 that contains a single insertion, a single deletion, a single substitution, multiple insertions, multiple deletions, multiple substitutions, or any combination thereof (e.g., single deletion together with multiple insertions). The invention provides multiple examples of isolated nucleic acid containing a nucleic acid sequence encoding an amino acid sequence having a variation of an amino acid sequence depicted in Figure 4, 9, 12, or 30.

Figure 6 depicts the amino acid sequence depicted in Figure 4 (designated STdxsp) aligned with 20 other amino acid sequences. Examples of variations of the STdxsp sequence include, without limitation, any variation of the STdxsp sequence provided in Figure 6. Such variations are provided in Figure 6 in that a comparison of the amino acid residue (or lack thereof) at a particular position of the STdxsp sequence with the amino acid residue (or lack thereof) at the same position of any of the other 20 amino acid sequences depicted in Figure 6 provides a list of specific changes for the STdxsp sequence. For example, the "t" at position 1148 of the STdxsp sequence can be substituted with an "s" as indicated in Figure 6. As also indicated in Figure 6, the "f" at position 575 of the STdxsp sequence can be substituted with an "m", "a", "l", "i", "y", or "v". For Figure 6, the nucleic acid numbering of Figure 2 is used to number the amino acid residue positions of the STdxsp sequence. Thus, the first amino acid residue of the STdxsp sequence starts with number 182 and proceeds in increments of three. It will be appreciated that the STdxsp sequence can contain any number of variations as well as any combination of types of variations. For example, the STdxsp sequence can contain one variation provided in Figure 6 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 6. It is noted that the 21 full-length amino acid sequences depicted in Figure 6 can be polypeptides having DXS activity.

Figure 14 depicts the amino acid sequence depicted in Figure 9 (designated RSddsp) and the amino acid sequence depicted in Figure 12 (designated STddsp) aligned with each other as well as aligned with three other amino acid sequences. For Figure 14, the nucleic acid numbering of Figure 7 is used to number the amino acid residue positions of the RSddsp sequence, and the nucleic acid numbering of Figure 10 is used to number

the amino acid residue positions of the STddsp sequence. Thus, the first amino acid residue of the RSddsp and STddsp sequences each start with a number other than 1 and proceed in increments of three. Examples of variations of the RSddsp sequence include, without limitation, any variation of the RSddsp sequence provided in Figure 14. Examples of variations of the STddsp sequence include, without limitation, any variation of the STddsp sequence provided in Figure 14. Such variations are provided in Figure 14 in that a comparison of the amino acid residue (or lack thereof) at a particular position of the RSddsp sequence or the STddsp sequence with the amino acid residue (or lack thereof) at the same position of any of the other amino acid sequences depicted in Figure 14 provides a list of specific changes for the RSddsp sequence and the STddsp sequence. For example, the "l" at position 762 of the RSddsp sequence or the "l" at position 1007 of the STddsp sequence can be substituted with an "a" as indicated in Figure 14. Again, it will be appreciated that the RSddsp sequence as well as the STddsp sequence can contain any number of variations as well as any combination of types of variations. For example, the RSddsp sequence can contain one variation provided in Figure 14 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 14. Likewise, the STddsp sequence can contain one variation provided in Figure 14 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 14. It is noted that the five full-length amino acid sequences depicted in Figure 14 can be polypeptides having DDS activity.

The amino acid sequence depicted in Figure 9 represents a *R. sphaeroides* (ATCC 17023) polypeptide having DDS activity. Another variant of this amino acid sequence is the amino acid sequence encoded by a clone isolated from *R. sphaeroides* (ATCC 35053). Briefly, a *R. sphaeroides* (ATCC 35053) clone was identified and found to encode an amino acid sequence identical to the amino acid sequence depicted in Figure 9 with the following exception. The *R. sphaeroides* (ATCC 35053) clone has a "y" at position 172 rather than an "h".

Figure 32 depicts the amino acid sequence depicted in Figure 30 (designated Stdxrp) aligned with 15 other amino acid sequences. Examples of variations of the Stdxrp sequence include, without limitation, any variation of the Stdxrp sequence provided in Figure 32. Such variations are provided in Figure 32 in that a comparison of

the amino acid residue (or lack thereof) at a particular position of the Stdxrp sequence with the amino acid residue (or lack thereof) at the same position of any of the other 15 amino acid sequences depicted in Figure 32 provides a list of specific changes for the Stdxrp sequence. It will be appreciated that the Stdxrp sequence can contain any number of variations as well as any combination of types of variations. For example, the Stdxrp sequence can contain one variation provided in Figure 32 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 32. It is noted that the full-length amino acid sequences depicted in Figure 32 can be polypeptides having DXR activity.

The invention also provides isolated nucleic acid containing a nucleic acid sequence encoding an amino acid sequence that contains a variant of a portion of the amino acid sequence depicted in Figure 4, 9, 12, or 30 as described herein.

2. Polypeptides

The invention provides substantially pure polypeptides. The term "substantially pure" as used herein with reference to a polypeptide means the polypeptide is substantially free of other polypeptides, lipids, carbohydrates, and nucleic acid with which it is naturally associated. Thus, a substantially pure polypeptide is any polypeptide that is removed from its natural environment and is at least 60 percent pure. A substantially pure polypeptide can be at least about 65, 70, 75, 80, 85, 90, 95, or 99 percent pure. Typically, a substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel.

Any substantially pure polypeptide having an amino acid sequence encoded by a nucleic acid within the scope of the invention is itself within the scope of the invention. In addition, any substantially pure polypeptide containing an amino acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:3 over that length as determined herein is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 641, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 641, and a Y coordinate greater than or equal to 50; where point C has an X coordinate

greater than or equal to 25, and a Y coordinate greater than or equal to 50; and where point D has an X coordinate greater than or equal to 5, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 641, 635, 630, 625, 620, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 641, 635, 630, 625, 620, or less; and the Y coordinate for point B can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (641, 100), point B can be (641, 95), point C can be (400, 95), and point D can be (400, 100).

Any substantially pure polypeptide containing an amino acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:39 over that length as determined herein is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 333, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 333, and a Y coordinate greater than or equal to 50; where point C has an X coordinate greater than or equal to 25, and a Y coordinate greater than or equal to 50; and where point D has an X coordinate greater than or equal to 5, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 333, 330, 325, 320, 315, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 333, 330, 325, 320, 315, or less; and the Y coordinate for point B can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (333, 100), point B can be (333, 95), point C can be (150, 95), and point D can be (150, 100).

Any substantially pure polypeptide containing an amino acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:42 over that length as determined herein is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 337, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 337, and a Y coordinate greater than or equal to 50; where point C has an X coordinate greater than or equal to 25, and a Y coordinate greater than or equal to 50; and where point D has an X coordinate greater than or equal to 5, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 337, 335, 330, 325, 320, 315, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 337, 335, 330, 325, 320, 315, or less; and the Y coordinate for point B can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, or more; and the Y coordinate for point C can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (337, 100), point B can be (337, 95), point C can be (150, 95), and point D can be (150, 100).

Any substantially pure polypeptide containing an amino acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:97 over that length as determined herein is within the scope of the invention provided the point defined by that length and percent identity is within the area defined by points A, B, C, and D of Figure 26; where point A has an X coordinate less than or equal to 386, and a Y coordinate less than or equal to 100; where point B has an X coordinate less than or equal to 386, and a Y coordinate greater than or equal to 50; where point C has an X coordinate greater than or equal to 25, and a Y coordinate greater than or equal to 50; and where point D has an X coordinate greater than or equal to 5, and a Y coordinate less than or equal to 100. For example, the X coordinate for point A can be 386, 380, 375, 370, 375, 360, 365, 350, 325, 300, or less; and the Y coordinate for point A can be 100, 99, 95, 90, 85, 80, 75, or less. The X coordinate for point B can be 386, 380, 375, 370, 375, 360,

365, 350, 325, 300, or less; and the Y coordinate for point B can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point C can be 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 350, or more; and the Y coordinate for point C can be 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or more. The X coordinate for point D can be 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, 200, 300, 350, or more; and the Y coordinate for point D can be 100, 99, 95, 90, 85, 80, 75, or less. In one embodiment, point A can be (386, 100), point B can be (386, 95), point C can be (350, 95), and point D can be (350, 100).

Any method can be used to obtain a substantially pure polypeptide. For example, common polypeptide purification techniques such as affinity chromotography and HPLC as well as polypeptide synthesis techniques can be used. In addition, any material can be used as a source to obtain a substantially pure polypeptide. For example, tissue from wild-type or transgenic animals can be used as a source material. In addition, tissue culture cells engineered to over-express a particular polypeptide of interest can be used to obtain substantially pure polypeptide. Further, a polypeptide within the scope of the invention can be "engineered" to contain an amino acid sequence that allows the polypeptide to be captured onto an affinity matrix. For example, a tag such as c-myc, hemagglutinin, polyhistidine, or Flag™ tag (Kodak) can be used to aid polypeptide purification. Such tags can be inserted anywhere within the polypeptide including at either the carboxyl or amino termini. Other fusions that could be useful include enzymes that aid in the detection of the polypeptide, such as alkaline phosphatase.

The invention provides polypeptides that contain the entire amino acid sequence depicted in Figure 4, 9, 12, or 30. In addition, the invention provides polypeptides that contain a portion of the amino acid sequence depicted in Figure 4, 9, 12, or 30. For example, the invention provides polypeptides that contain a 15 amino acid sequence identical to any 15 amino acid sequence depicted in Figure 4, 9, 12, or 30 including, without limitation, the sequence starting at amino acid residue number 1 and ending at amino acid residue number 15, the sequence starting at amino acid residue number 2 and ending at amino acid residue number 16, the sequence starting at amino acid residue number 3 and ending at amino acid residue number 17, and so forth. It will be appreciated that the invention also provides polypeptides that contain an amino acid

sequence that is greater than 15 amino acid residues (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more amino acid residues) in length and identical to any portion of the sequence depicted in Figure 4, 9, 12, or 30. For example, the invention provides polypeptides that contain a 25 amino acid sequence identical to any 25 amino acid sequence depicted in Figure 4, 9, 12, or 30 including, without limitation, the sequence starting at amino acid residue number 1 and ending at amino acid residue number 25, the sequence starting at amino acid residue number 2 and ending at amino acid residue number 26, the sequence starting at amino acid residue number 3 and ending at amino acid residue number 27, and so forth. Additional examples include, without limitation, polypeptides that contain an amino acid sequence that is 50 or more amino acid residues (e.g., 100, 150, 200, 250, 300, 350, or more amino acid residues) in length and identical to any portion of the sequence depicted in Figure 4, 9, 12, or 30. Such polypeptides can include, without limitation, those polypeptides containing a amino acid sequence represented in a single line of sequence depicted in Figure 4, 9, 12, or 30 since each line of sequence depicted in these figures, with the possible exception of the last line, provides a 50 amino acid sequence.

In addition, the invention provides polypeptides that an amino acid sequence having a variation of the amino acid sequence depicted in Figure 4, 9, 12, or 30. For example, the invention provides polypeptides containing an amino acid sequence depicted in Figure 4, 9, 12, or 30 that contains a single insertion, a single deletion, a single substitution, multiple insertions, multiple deletions, multiple substitutions, or any combination thereof (e.g., single deletion together with multiple insertions). The invention provides multiple examples of polypeptides containing an amino acid sequence having a variation of an amino acid sequence depicted in Figure 4, 9, 12, or 30.

Figure 6 depicts the amino acid sequence depicted in Figure 4 (designated STdxsp) aligned with 20 other amino acid sequences. Examples of variations of the STdxsp sequence include, without limitation, any variation of the STdxsp sequence provided in Figure 6. Such variations are provided in Figure 6 in that a comparison of the amino acid residue (or lack thereof) at a particular position of the STdxsp sequence with the amino acid residue (or lack thereof) at the same position of any of the other 20 amino acid sequences depicted in Figure 6 provides a list of specific changes for the STdxsp

sequence. For example, the "t" at position 1148 of the STdxsp sequence can be substituted with an "s" as indicated in Figure 6. As also indicated in Figure 6, the "f" at position 575 of the STdxsp sequence can be substituted with an "m", "a", "l", "i", "y", or "v". For Figure 6, the nucleic acid numbering of Figure 2 is used to number the amino acid residue positions of the STdxsp sequence. Thus, the first amino acid residue of the STdxsp sequence starts with number 182 and proceeds in increments of three. It will be appreciated that the STdxsp sequence can contain any number of variations as well as any combination of types of variations. For example, the STdxsp sequence can contain one variation provided in Figure 6 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 6. It is noted that the 21 full-length amino acid sequences depicted in Figure 6 can be polypeptides having DXS activity.

Figure 14 depicts the amino acid sequence depicted in Figure 9 (designated RSddsp) and the amino acid sequence depicted in Figure 12 (designated STddsp) aligned with each other as well as aligned with three other amino acid sequences. For Figure 14, the nucleic acid numbering of Figure 7 is used to number the amino acid residue positions of the RSddsp sequence, and the nucleic acid numbering of Figure 10 is used to number the amino acid residue positions of the STddsp sequence. Thus, the first amino acid residue of the RSddsp and STddsp sequences each start with a number other than 1 and proceed in increments of three. Examples of variations of the RSddsp sequence include, without limitation, any variation of the RSddsp sequence provided in Figure 14. Examples of variations of the STddsp sequence include, without limitation, any variation of the STddsp sequence provided in Figure 14. Such variations are provided in Figure 14 in that a comparison of the amino acid residue (or lack thereof) at a particular position of the RSddsp sequence or the STddsp sequence with the amino acid residue (or lack thereof) at the same position of any of the other amino acid sequences depicted in Figure 14 provides a list of specific changes for the RSddsp sequence and the STddsp sequence. For example, the "l" at position 762 of the RSddsp sequence or the "l" at position 1007 of the STddsp sequence can be substituted with an "a" as indicated in Figure 14. Again, it will be appreciated that the RSddsp sequence as well as the STddsp sequence can contain any number of variations as well as any combination of types of variations. For example, the RSddsp sequence can contain one variation provided in Figure 14 or more than one

(e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 14. Likewise, the STddsp sequence can contain one variation provided in Figure 14 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 14. It is noted that the five full-length amino acid sequences depicted in Figure 14 can be polypeptides having DDS activity.

Figure 32 depicts the amino acid sequence depicted in Figure 30 (designated Stdxrp) aligned with 15 other amino acid sequences. Examples of variations of the Stdxrp sequence include, without limitation, any variation of the Stdxrp sequence provided in Figure 32. Such variations are provided in Figure 32 in that a comparison of the amino acid residue (or lack thereof) at a particular position of the Stdxrp sequence with the amino acid residue (or lack thereof) at the same position of any of the other 15 amino acid sequences depicted in Figure 32 provides a list of specific changes for the Stdxrp sequence. It will be appreciated that the Stdxrp sequence can contain any number of variations as well as any combination of types of variations. For example, the Stdxrp sequence can contain one variation provided in Figure 32 or more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, or more) of the variations provided in Figure 32. It is noted that the full-length amino acid sequences depicted in Figure 32 can be polypeptides having DXR activity.

The invention also provides polypeptides containing an amino acid sequence that contains a variant of a portion of the amino acid sequence depicted in Figure 4, 9, 12, or 30 as described herein.

3. Genetically modified cells

Any cell containing an isolated nucleic acid within the scope of the invention is itself within the scope of the invention. This includes, without limitation, prokaryotic cells such as cells from the Rhodospirillaceae family (e.g., *Rhodobacter* cells) and eukaryotic cells such as plant and mammalian cells. It is noted that cells containing an isolated nucleic acid of the invention are not required to express the isolated nucleic acid. In addition, the isolated nucleic acid can be integrated into the genome of the cell or maintained in an episomal state. In other words, cells can be stably or transiently transformed with an isolated nucleic acid of the invention.

Any method can be used to introduce an isolated nucleic acid into a cell. In fact, many methods for introducing nucleic acid into a cell, whether *in vivo* or *in vitro*, are well known to those skilled in the art. For example, calcium phosphate precipitation, electroporation, heat shock, lipofection, microinjection, conjugation, and viral-mediated nucleic acid transfer are common methods that can be used to introduce nucleic acid into a cell. In addition, naked DNA can be delivered directly to cells *in vivo* as describe elsewhere (U.S. Patent Number 5,580,859 and U.S. Patent Number 5,589,466 including continuations thereof). Further, nucleic acid can be introduced into cells by generating transgenic animals.

Any method can be used to identify cells that contain an isolated nucleic acid within the scope of the invention. For example, PCR and nucleic acid hybridization techniques such as Northern and Southern analysis can be used. In some cases, immunohistochemistry and biochemical techniques can be used to determine if a cell contains a particular nucleic acid by detecting the expression of a polypeptide encoded by that particular nucleic acid. For example, detection of polypeptide X-immunoreactivity after introduction of an isolated nucleic acid containing a cDNA that encodes polypeptide X into a cell that does not normally express polypeptide X can indicate that that cell not only contains the introduced nucleic acid but also expresses the encoded polypeptide X from that introduced nucleic acid. In this case, the detection of any enzymatic activities of polypeptide X also can indicate that that cell contains the introduced nucleic acid and expresses the encoded polypeptide X from that introduced nucleic acid.

Any method can be used to direct the expression of an amino acid sequence from a nucleic acid. Such methods are well known to those skilled in the art, and include, without limitation, constructing a nucleic acid such that a regulatory element drives the expression of a nucleic acid sequence that encodes a polypeptide. Typically, regulatory elements are DNA sequences that regulate the expression of other DNA sequences at the level of transcription. Such regulatory elements include, without limitation, promoters, enhancers, and the like. In addition, any method for expressing a polypeptide from an exogenous nucleic acid molecule in microorganisms such as bacteria and yeast can be used. For example, well-known methods for making and using nucleic acid constructs that are capable of expressing exogenous polypeptides within *Rhodobacter* species (e.g.,

R. sphaeroides and *R. capsulatus*) can be used. *See, e.g.*, Dryden and Dowhan, *J. Bacteriol.*, 178(4):1030-1038 (1996); Vasilyeva *et al.*, *Applied Biochemistry and Biotechnology*, 77-79:337-345 (1999); Graichen *et al.*, *J. Bacteriol.*, 181(14):4216-4222 (1999); Johnson *et al.*, *J. Bacteriol.*, 167(2):604-610 (1986); and Duport *et al.*, *Gene*, 145:103-108 (1994). Further, any methods can be used to identify cells that express an amino acid sequence from a nucleic acid. Such methods are well known to those skilled in the art, and include, without limitation, immunocytochemistry, Western analysis, Northern analysis, and RT-PCR.

The cells described herein can contain a single copy, or multiple copies (e.g., about 5, 10, 20, 35, 50, 75, 100 or 150 copies), of a particular exogenous nucleic acid. For example, a bacterial cell can contain about 50 copies of exogenous nucleic acid X. In addition, the cells described herein can contain more than one particular exogenous nucleic acid. For example, a bacterial cell can contain about 50 copies of exogenous nucleic acid X as well as about 75 copies of exogenous nucleic acid Y. In these cases, each different nucleic acid can encode a different polypeptide having its own unique enzymatic activity. For example, a bacterial cell can contain two different exogenous nucleic acids such that a high level of CoQ(10) is produced. In this example, such a cell can contain a first exogenous nucleic acid that encodes a polypeptide having DXS activity and a second exogenous nucleic acid that encodes a polypeptide having DDS activity. In addition, a single exogenous nucleic acid can encode one or more than one polypeptide. For example, a single nucleic acid can contain sequences that encode three different polypeptides.

In addition to providing cells that contain an isolated nucleic acid of the invention, the invention provides cells (e.g., plant cells, animal cells, and microorganisms) that can be used to produce an isoprenoid compound such as CoQ(10). The term "microorganism" as used herein refers to all microscopic organisms including, without limitation, bacteria, algae, fungi, and protozoa. It is noted that bacteria cells can be membraneous bacteria or non-membraneous bacteria.

The term "non-membraneous bacteria" as used herein refers to any bacteria lacking intracytoplasmic membrane. The term "membraneous bacteria" as used herein refers to any naturally-occurring, genetically modified, or environmentally modified

bacteria having an intracytoplasmic membrane. An intracytoplasmic membrane can be organized in a variety of ways including, without limitation, vesicles, tubules, thylakoid-like membrane sacs, and highly organized membrane stacks. Any method can be used to analyze bacteria for the presence of intracytoplasmic membranes including, without limitation, electron microscopy, light microscopy, and density gradients. *See, e.g.*, Chory *et al.*, *J. Bacteriol.*, 159:540-554 (1984); Niederman and Gibson, Isolation and Physiochemical Properties of Membranes from Purple Photosynthetic Bacteria. In: The Photosynthetic Bacteria, Ed. By Roderick K. Clayton and William R. Sistrom, Plenum Press, pp. 79-118 (1978); and Lueking *et al.*, *J. Biol. Chem.*, 253: 451-457 (1978). Examples of membraneous bacteria that can be used herein include, without limitation, bacteria of the Rhodospirillaceae family such as those in the genus Rhodobacter (e.g., *R. sphaeroides*, *R. capsulatus*, *R. sulfidophilus*, *R. adriaticus*, and *R. veldkampii*), the genus Rhodospirillum (e.g., *R. rubrum*, *R. photometricum*, *R. molischianum*, *R. fulvum*, and *R. salinarum*), the genus Rhodopseudomonas (e.g., *R. palustris*, *R. viridis*, and *R. sulfoviridis*), the genus Rhodomicrobium, the genus Rhodocyclus, and the genus Rhodopila; bacteria of the Chromatiaceae family such as those in the genus Chromatium, genus Thiocystis, the genus Thiospirillum, the genus Thiocapsa, the genus Lamprobacter, the genus Lalmprocystis, the genus Thiodictyon, the genus Amoebobacter, and the genus Thiopedia; green sulfur bacteria such as those in the genus Chlorobium and the genus Prosthecochloris; bacteria of the Methylococcaceae family such as those in the genus Methylococcus (e.g., *M. capsulatus*), and the genus Methylomonas (e.g., *M. methanica*); and particular bacteria of the Nitrobacteraceae family such as those in the genus Nitrobacter (e.g., *N. winogradsky* and *N. hamburgensis*), the genus Nitrococcus (e.g., *N. mobilis*), and the genus Nitrosomonas (e.g., *N. europaea*).

Membraneous bacteria can be highly membraneous bacteria. The term "highly membraneous bacteria" as used herein refers to any bacterium having more intracytoplasmic membrane than *R. sphaeroides* (ATCC 17023) cells have after the *R. sphaeroides* (ATCC 17023) cells have been (1) cultured chemoheterotrophically under aerobic conditions for four days, (2) cultured chemoheterotrophically under oxygen-limited conditions for four hours, and (3) harvested. The aerobic culture conditions involve culturing the cells in the dark at 30°C in the presence of 25 percent oxygen. The

oxygen-limited conditions involve culturing the cells in the light at 30°C in the presence of 2 percent oxygen. After the four hour culturing step under oxygen-limited conditions, the *R. sphaeroides* (ATCC 17023) cells are harvested by centrifugation and analyzed.

Typically, any cell (e.g., membraneous bacteria) can be genetically modified such that a particular isoprenoid compound is produced. Such cells can contain exogenous nucleic acid that encodes a polypeptide having enzymatic activity. For example, a microorganism having endogenous DDS activity can be transformed with an exogenous nucleic acid that encodes a polypeptide having DDS activity. In this case, the microorganism can have increased DDS activity which can lead to an increased production of CoQ(10). Thus, a cell can be given an exogenous nucleic acid that encodes a polypeptide having an enzymatic activity that catalyzes the production of a compound normally produced by that cell. In this case, the genetically modified cell can produce more of the compound, or can produce the compound more efficiently, than a similar cell not having the genetic modification. Alternatively, a cell can be given an exogenous nucleic acid that encodes a polypeptide having an enzymatic activity that catalyzes the production of a compound that is not normally produced by that cell.

The invention provides cells containing exogenous nucleic acid that encodes a polypeptide having enzymatic activity that leads to an increased production of CoQ(10). Such cells can contain nucleic acid that encodes a polypeptide having DDS activity. Other examples include, without limitation, cells containing exogenous nucleic acid that encodes polypeptides having DXS, ODS, SDS, DXR, 4-diphosphocytidyl-2C-methyl-D-erythritol synthase (e.g., ispD), 4-diphosphocytidyl-2C-methyl-D-erythritol kinase (e.g., ispE), and/or chorismate lyase (e.g., ubiC) activity. Nucleic acid molecules that encode polypeptides having such enzymatic activities can be obtained as described herein. For example, nucleic acid encoding a polypeptide having chorismate lyase can be cloned using the sequence information provided in Genbank® accession number X66619.

Typically, microorganisms of the invention produce CoQ(10) with the yield (mg of CoQ(10) per g of dry biomass) being at least about 5 (e.g., at least about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, or more) percent greater than that of a comparable wild-type strain grown under similar conditions. Bacteria can produce more CoQ(10) when grown under anaerobic conditions as compared to aerobic conditions. For example,

anaerobically cultured bacteria can produce about 3 to 4 fold more CoQ(10) than aerobically cultured bacteria of the same species. When determining the yield of isoprenoid compound production for a particular cell (e.g., microorganism), any method can be used. *See, e.g.*, Cohen-Bazire *et al.*, *J. Cell Comp. Physiol.*, 49:25-68 (1957); Edlund, *J. Chromatogr.*, 425:87-97 (1988); Rousseau and Varin, *J. Chromatogr. Sci.*, 36:247-52 (1998); and Leray *et al.*, *J. Lipid Res.*, 39:2099-2105 (1998).

The invention provides a cell containing an exogenous nucleic acid that encodes a polypeptide having DXS, DDS, ODS, SDS, DXR, 4-diphosphocytidyl-2C-methyl-D-erythritol synthase (e.g., ispD), 4-diphosphocytidyl-2C-methyl-D-erythritol kinase (e.g., ispE), and/or chorismate lyase (e.g., ubiC) activity. Nucleic acid molecules that encode polypeptides having such enzymatic activities can be obtained as described herein. The invention also provides a cell that contains more than one different exogenous nucleic acid molecule with each different exogenous nucleic acid molecule encoding a polypeptide having a different one of the following enzymatic activities: DXS, DDS, ODS, SDS, DXR, 4-diphosphocytidyl-2C-methyl-D-erythritol synthase (e.g., ispD), 4-diphosphocytidyl-2C-methyl-D-erythritol kinase (e.g., ispE), and/or chorismate lyase (e.g., ubiC) activity. For example, the invention provides a cell containing a first exogenous nucleic acid encoding a polypeptide having DXS activity and a second exogenous nucleic acid encoding a polypeptide having DDS activity.

The invention provides a cell containing an exogenous nucleic acid containing a dxs sequence (e.g., Stdxs sequence), dds sequence (e.g., Stdds or Rsdds sequence), dxr sequence (e.g., Stdxr sequence), ubiC sequence (e.g., EcUbiC sequence), or lytB sequence (e.g., RsLytB sequence). Such nucleic acids can be obtained as described herein. The invention also provides a cell that contains more than one of the following sequences: a dxs sequence (e.g., Stdxs sequence), dds sequence (e.g., Stdds or Rsdds sequence), dxr sequence (e.g., Stdxr sequence), ubiC sequence (e.g., EcUbiC sequence), or lytB sequence (e.g., RsLytB sequence). For example, the invention provides a cell containing a first exogenous nucleic acid containing a dds sequence and a second exogenous nucleic acid containing a dxs sequence. Likewise, the invention provides a cell containing a single exogenous nucleic acid that contains a dds sequence and a dxs sequence.

Typically, a microorganism within the scope of the invention catabolizes a hexose carbon such as glucose. A microorganism, however, can catabolize a pentose carbon (e.g., ribose, arabinose, xylose, and lyxose). In other words, a microorganism within the scope of the invention can either utilize hexose or pentose carbon. In addition, a microorganism within the scope of the invention can use carbon sources such as methanol and/or organic acids (e.g., succinic acid or malic acid).

Any cells described herein can have reduced enzymatic activity such as reduced geranylgeranyl pyrophosphate synthase and/or magnesium protoporphyrin IX chelatase activity. Any cell described herein can have reduced biological activity such as reduced activity of aerobic repressor polypeptides (e.g., PPSR) or oxidation-reduction sensor polypeptides (e.g., CBB3). In the case of multi-subunit molecules such as CBB3, the activity of the oxidation-reduction sensor polypeptide can be reduced by inactivating one or more than one of the subunits. For example, CBB3 activity can be reduced by inactivating a single subunit of CBB3 such as the ccoN subunit.

The term "reduced" as used herein with respect to a cell and a particular activity (e.g., particular enzymatic activity) refers to a lower level of activity than that measured in a comparable cell of the same species. Thus, a *R. sphaeroides* cell lacking geranylgeranyl pyrophosphate synthase activity is considered to have reduced geranylgeranyl pyrophosphate synthase activity since most, if not all, comparable *R. sphaeroides* cells have at least some geranylgeranyl pyrophosphate synthase activity. Such reduced enzymatic activities can be the result of lower enzyme concentration, lower specific activity of an enzyme, or combinations thereof.

Many different methods can be used to make a cell having reduced enzymatic and/or biological activity. For example, a *R. sphaeroides* cell can be engineered to have a disrupted enzyme-encoding locus using common mutagenesis or knock-out technology. Alternatively, antisense technology can be used to reduce enzymatic activity. For example, a *R. sphaeroides* cell can be engineered to contain a cDNA that encodes an antisense molecule that prevents an enzyme from being made. The term "antisense molecule" as used herein encompasses any nucleic acid that contains sequences that correspond to the coding strand of an endogenous polypeptide. An antisense molecule also can have flanking sequences (e.g., regulatory sequences). Thus, antisense molecules

can be ribozymes or antisense oligonucleotides. A ribozyme can have any general structure including, without limitation, hairpin, hammerhead, or axhead structures, provided the molecule cleaves RNA.

Cells having a reduced enzymatic and/or biological activity can be identified using any method. For example, a *R. sphaeroides* cell having reduced geranylgeranyl pyrophosphate synthase activity can be easily identified using common biochemical methods that measure geranylgeranyl pyrophosphate synthase activity. *See, e.g.*, Math *et al.*, *Proc. Natl. Acad. Sci. USA*, 89(15):6761-6764 (1992).

The invention provides a cell containing reduced geranylgeranyl diphosphate synthase, aerobic repressor, and/or cbb3-type cytochrome oxidase activity. Such cells can have reduced geranylgeranyl diphosphate synthase, aerobic repressor, and/or cbb3-type cytochrome oxidase activity as a result of disrupting the endogenous sequences that encode polypeptides having these activities. For example, a cell can have reduced geranylgeranyl diphosphate synthase activity as a result of knocking out a portion of the endogenous crtE sequence within a cell's genome; a cell can have reduced aerobic repressor activity as a result of knocking out a portion of the endogenous ppsR sequence within a cell's genome; and a cell can have reduced cbb3-type cytochrome oxidase activity as a result of knocking out a portion of the endogenous ccoN sequence within a cell's genome.

The invention also provides a cell containing non-functional crtE, ppsR, and/or ccoN nucleic acid sequences within its genome such that the encoded polypeptide is either mutated or not expressed. Such cells can be used to produce large amounts of CoQ(10). The sequence of crtE can be as set forth in Genbank® accession number AJ010302. The sequence of ppsR can be as set forth in Genbank® accession number AJ010302 or L19596. The sequence of ccoN can be as set forth in Genbank® accession number U58092. Knockout technology can be used to make cells containing non-functional crtE, ppsR, and/or ccoN nucleic acid sequences.

4. Producing isoprenoid compounds

The cells described herein can be used to produce isoprenoid compounds. For example, a microorganism having endogenous DDS activity can be transformed with

nucleic acid that encodes a polypeptide having DDS activity such that the microorganism produces more CoQ(10) than had the microorganism not been given that nucleic acid. Once transformed, the microorganism can be used cultured under conditions optimal for CoQ(10) production.

In addition, substantially pure polypeptides having enzymatic activity can be used alone or in combination with cells to produce isoprenoid compounds. For example, a preparation containing a substantially pure polypeptide having DDS activity can be used to catalyze the formation of CoQ(10). Further, cell-free extracts containing a polypeptide having enzymatic activity can be used alone or in combination with substantially pure polypeptides and/or cells to produce isoprenoid compounds. For example, a cell-free extract containing a polypeptide having DXS activity can be used to form 1-deoxyxyulose-5-phosphate, while a microorganism containing polypeptides have the enzymatic activities necessary to catalyze the reactions needed to form CoQ(10) from 1-deoxyxyulose-5-phosphate can be used to produce CoQ(10). Any method can be used to produce a cell-free extract. For example, osmotic shock, sonication, and/or a repeated freeze-thaw cycle followed by filtration and/or centrifugation can be used to produce a cell-free extract from intact cells.

It is noted that a cell, substantially pure polypeptide, and/or cell-free extract can be used to produce a particular isoprenoid compound that is, in turn, treated chemically to produce another compound. For example, a microorganism can be used to produce CoQ(10), while a chemical process is used to modify CoQ(10) into a CoQ(10) derivative such as CoQ10 containing a polar group. Likewise, a chemical process can be used to produce a particular compound that is, in turn, converted into an isoprenoid compound using a cell, substantially pure polypeptide, and/or cell-free extract described herein. For example, a chemical process can be used to produce deoxyxylose-5-phosphate, while a microorganism can be used convert deoxyxylose-5-phosphate into CoQ(10).

Typically, a particular isoprenoid compound is produced by providing a microorganism and culturing the provided microorganism with culture medium such that that isoprenoid compound is produced. In general, the culture media and/or culture conditions can be such that the microorganisms grow to an adequate density and produce the desired compound efficiently. For large-scale production processes, the following

methods can be used. First, a large tank (e.g., a 100 gallon, 200 gallon, 500 gallon, or more tank) containing appropriate culture medium with, for example, a glucose carbon source is inoculated with a particular microorganism. After inoculation, the microorganisms are incubated to allow biomass to be produced. Once a desired biomass is reached, the broth containing the microorganisms can be transferred to a second tank. This second tank can be any size. For example, the second tank can be larger, smaller, or the same size as the first tank. Typically, the second tank is larger than the first such that additional culture medium can be added to the broth from the first tank. In addition, the culture medium within this second tank can be the same as, or different from, that used in the first tank. For example, the first tank can contain medium with xylose, while the second tank contains medium with glucose.

Once transferred, the microorganisms can be incubated to allow for the production of the desired isopreniod compound. Once produced, any method can be used to isolate the desired compound. For example, if the microorganism releases the desired isoprenoid compound into the broth, then common separation techniques can be used to remove the biomass from the broth, and common isolation procedures (e.g., extraction, distillation, and ion-exchange procedures) can be used to obtain the isoprenoid compound from the microorganism-free broth. In addition, the desired isoprenoid compound can be isolated while it is being produced, or it can be isolated from the broth after the product production phase has been terminated. If the microorganism retains the desired isoprenoid compound, then the biomass can be collected and treated to release the isoprenoid compound, and the released isoprenoid compound can be isolated.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1 – Cloning nucleic acid that encodes a *Sphingomonas trueperi* polypeptide having DXS activity

S. trueperi cells were obtained from the American Type Culture Collection (ATCC Cat. No. 12417). To isolate bacterial genomic DNA, cells were grown in 100-200 mL cultures for 2-3 days at 30°C on a shaker rotating at 250 rpm. Cultured cells

were centrifuged to form a cell pellet, washed by resuspending the pellet in a solution of 10 mM Tris/1 mM EDTA, and centrifuged again as before. The cell pellets were resuspended in 5 mL of GTE buffer per 100 mL of original culture. GTE buffer is 50 mM glucose/25 mM Tris-HCl (pH 8.0)/10 mM EDTA (pH 8.0). The bacterial cell walls were lysed by adding lysozyme (final concentration of 1 mg/mL), Proteinase K (final concentration of 1 mg/mL), and mutanolysin (final concentration of 5.5 μg/mL) to the resuspended cell solution to form a lysing mixture that was incubated for 90 minutes at 37°C. After this incubation, sodium dodecyl sulfate was added to the mixture to a final concentration of 1 percent, and additional Proteinase K was added until the concentration in the solution was 2 mg/mL. After a 1 hour incubation at 50°C, the solution containing the lysed cells was diluted 1:1 with fresh GTE buffer. Once diluted, sodium chloride was added to the solution to a final concentration of 0.15 M. Polypeptides and molecules other than nucleic acids were removed from the lysed bacterial cell solution by adding an equal volume of an organic mixture made up of phenol, chloroform, and isoamyl alcohol at a ratio of 25:24:1 (hereinafter referred to as PCIA). After adding PCIA, the solution was mixed. To separate the organic phase from the DNA-containing aqueous phase, the mixture was centrifuged at 12,000 x g for 10 minutes. The aqueous phase was transferred to a clean tube and re-extracted with an equal volume of chloroform alone. The aqueous and organic phases were separated by centrifugation at 3,000 x g for 10 minutes. The aqueous phase was again removed to a new tube and treated with 2.5 mg of RNase to degrade any bacterial RNA present. The purified DNA was recovered by adding 2.5 volumes of ethanol to the aqueous phase. After mixing the solution, the precipitated DNA was removed by spooling it on a glass rod. The spooled DNA was rinsed with 70 percent ethanol. Once rinsed, the ethanol was allowed to evaporate by leaving the DNA exposed to the air until dry. The dried DNA was resuspended in a solution of 10 mM Tris (pH 8.5). The resuspended DNA was re-extracted with PCIA followed by chloroform alone as before. The DNA was re-precipitated by adding one-tenth volume of 7.5 M ammonium acetate and 2.5 volumes ethanol, followed by spooling, rinsing, and air drying. The purified DNA was resuspended in 10 mM Tris (pH 8.5).

The following polymerase chain reaction (PCR) procedure was used to isolate nucleic acid that encodes a *S. trueperi* polypeptide having DXS activity. Three

degenerate forward PCR primers (F1, F2, and F3) and three degenerate reverse PCR primers (R1, R2, and R3) were designed by comparing sequences of several clones that encode polypeptides have DXS activity (Figure 15). The sequence of each degenerate primer was as follows:

F1: 5'-RTKATTYTMAAYGAYAAYGAAATG-3' (SEQ ID NO:53)
F2: 5'-TTTGAAGARYTVGGYWTTAACTA-3' (SEQ ID NO:54)
F3: 5'-RCAYCARGCTTAYSCVCAYAA-3' (SEQ ID NO:55)
R1: 5'-CGTGYTGYTCDGCRATHGCBAC-3' (SEQ ID NO:56)
R2: 5'-TGYTCDGCRATHGCBACRTCRAA-3' (SEQ ID NO:57)
R3: 5'-GGSCCDATRTAGTTAAWRCC-3' (SEQ ID NO:58)

The primers were used in all logical combinations in PCR using Taq polymerase (Roche Molecular Biochemicals, Indianapolis, IN) and 1 ng of purified genomic DNA per microliter of reaction mix. Each PCR reaction was conducted using a touchdown PCR program with four cycles at each of the following annealing temperatures: 60°C, 58°C, 56°C, and 54°C, followed by 25 cycles at 52°C. Each cycle had an initial 30 second denaturing step at 94°C and a 90 second extension step at 72°C. The program had an initial denaturing step of 2 minutes at 94°C and final extension step of 5 minutes at 72°C.

Between about 2 μM and 12 μM of each PCR primer was used in each reaction, depending on the degree of degeneracy. After each PCR reaction was complete, a portion of each reaction was separated by gel electrophoresis using a 1.5 percent TAE (Tris-acetate-EDTA) agarose gel. The results from the gel electrophoresis indicated that the combination of degenerate primer F3 with degenerate primer R2 produced a nucleic acid molecule of 882 bp (referred to as the F3R2 fragment). The F3R2 fragment was purified away from the agarose gel matrix using the Qiagen Gel Extraction procedure according to the manufacturer's instructions (Qiagen Inc., Valencia, CA). A portion of the purified fragment was ligated into the pCRII-TOPO vector. The vector containing the F3R2 fragment was inserted into *E. coli* TOP10 cells using the TOPO cloning procedure (Invitrogen, Carlsbad, CA). The transformed TOP10 cells were plated onto LB agar plates containing 100 μg/mL of ampicillin (Amp) and 50 μg/mL of 5-Bromo-4-Chloro-3-Indolyl-β-D-Galactopyranoside (Xgal). Single white colonies were re-plated onto fresh

LB-Amp-Xgal plates and screened by PCR with the F3 and R2 primers to confirm the presence of plasmids with the desired insert. Plasmid DNAs were obtained from bacterial colonies using the QiaPrep Spin Miniprep Kit (Qiagen, Inc). The plasmid DNAs were then quantified and sequenced with the M13 forward and reverse primers. Sequence analysis indicated that the sequence of the F3R2 fragment aligned with sequences from other nucleic acid molecules that encode polypeptides having DXS activity.

To obtain the complete coding sequence for the *S. trueperi* polypeptide having DXS activity, genome walking was performed as follows. Primers were designed based upon the sequence of the 882 bp F3R2 fragment for walking in both the upstream and downstream directions. These walking primers had the following sequences:

GSP1F: 5'-TCGTGACCAAGAAGGGCAAGGGCTATG-3'(SEQ ID NO:59)
GSP2F: 5'-GACAAGTATCACGGCGTCCAGAAGTTC-3' (SEQ ID NO:60)
GSP1R: 5'-ATAGCCCTTGCCCTTCTTGGTCACGAC-3' (SEQ ID NO:61)
GSP2R: 5'-CGAACGGATCATACTCGCTCTCGCTG-3' (SEQ ID NO:62)

The GSP1F and GSP2F primers are primers that face downstream of the DXS polypeptide start codon, while the GSP1R and GSP2R primers are primers that face in the opposite direction. In addition, GSP2F and GSP2R are nested inside of the GSP1F and GSP1R primers. Genome walking was conducted according to the manual of CLONTECH's Universal Genome Walking kit (CLONTECH Laboratories, Inc., Palo Alto, CA) with the exception that *Fsp* I and *Sma* I were used instead of *Dra* I and *EcoR* V. The genomic DNA used was from *S. trueperi*. DMSO was added to the PCR mixture until a final concentration of 5 percent was reached. The PCR reactions were performed using a Perkin Elmer 9700 Thermocycler. The first round of PCR consisted of 7 cycles of 2 seconds at 94°C and 3 minutes at 72°C, followed by 36 cycles of 2 seconds at 94°C and 3 minutes at 67°C, with a final extension at 67°C for 4 minutes. The second round of PCR consisted of 5 cycles of 2 seconds at 94°C and 3 minutes at 72°C, followed by 24 cycles of 2 seconds at 94°C and 3 minutes at 67°C, with a final extension at 67°C for 4 minutes. After the PCR was complete, a portion of the reaction mix from each round was separated by gel electrophoresis using a 1.5 percent TAE agarose gel. Good

amplification products were obtained with the *Pvu* II and *Stu* I libraries using the GSP1F and GSP2F primers and with the *Fsp* I and *Pvu* II libraries using the GSP1R and GSP2R primers. The second round products from each of these libraries were gel purified, cloned using the TOPO cloning procedure (Invitrogen, Carlsbad, CA), and sequenced. A 1.7 kilobase (kb) fragment was subcloned from the *Pvu* IIF library, a 2.8 kb fragment was subcloned from the *Stu* IF library, a 400 bp fragment was subcloned from the *Fsp* IR library, and a 330 bp fragment was subcloned from the *Pvu* IIR library. Each of these subcloned fragments was sequenced. Sequence analysis indicated that each subcloned fragment contained a sequence that overlapped with that of the F3R2 fragment and was similar to other nucleic acid sequences that encode polypeptides having DXS activity.

Because the sequence information obtained by genome walking extended 13 bp upstream of the translational start codon, a second genome walk was conducted to gain additional sequence information. This second walk used GSPB2R, 5'-TGAGGATCTTGTGCGGATAGC-ATTGGTG-3' (SEQ ID NO:63) as the first round primer and GSPB3R, 5'-AGCGGCGTCTTG-GGTAGGTCAGCCAT-3' (SEQ ID NO:64) as the second round primer. The second walk was conducted using only the *Sma* I and *Stu* I libraries. CLONTECH's Advantage-GC Genomic Polymerase was used for PCR with a 1.0 mM GC Melt concentration according to the manufacturer's specifications. The first round of PCR was conducted using a Perkin Elmer 9700 Thermocycler with an initial denaturing step at 96°C for 5 seconds followed by 7 cycles consisting of 2 seconds at 94°C and 3 minutes at 72°C, followed by 36 cycles consisting of 2 seconds at 94°C and 3 minutes at 66°C, with a final extension at 66°C for 4 minutes. The second round of PCR had 5 cycles consisting of 2 seconds at 94°C and 3 minutes at 72°C, followed by 26 cycles consisting of 2 seconds at 94°C and 3 minutes at 66°C, with a final extension at 66°C for 4 minutes. Portions of the PCR products from each round were separated by gel electrophoresis using a 1.5 percent TAE agarose gel. The gel electrophoresis revealed the presence of a 250 bp amplification product obtained from the second round of PCR using the *Stu* I library. This fragment was gel purified, cloned using the TOPO cloning procedure (Invitrogen, Carlsbad, CA), and sequenced. An overlap with the previously obtained sequence was found, extending the length of the clone to 181 bp before the start codon. The full-length clone containing coding and non-

coding sequence was 3626 bp in length (Figure 2). The open reading frame was 1926 bp in length (Figure 3), which encoded a polypeptide with 641 amino acid residues (Figure 4).

The coding sequence of the DXS polypeptide was amplified by PCR using *S. trueperi* genomic DNA as template. Primers were designed based on the sequence obtained above. The sequences of the primers were as follows:

SHDXF1: 5'-ATATGGTACCGTGTGACTGACCTGTCCAAC-3' (SEQ ID NO:65)
SHDXR1: 5'-AGTCTCTAGAATGTTGGAGATTCAAGGTGG-3' (SEQ ID NO:66)

These primers were designed to introduce a *Kpn* I restriction site at the beginning of the amplified fragment and an *Xba* I restriction site at the end of the amplified fragment. The sequence of each restriction site is underlined. The PCR reaction mix contained the following: 100 ng genomic DNA, 2 μL of each primer (SHDXF1 and SHDXR1, each at 50 μM), 10 μL 10X *Pfu* Plus buffer, 5 μL DMSO, 8 μL dNTPs (10 μM each) and 5 units *Pfu* polymerase in a final volume of 100 μL. Each PCR reaction was performed in a Perkin Elmer Geneamp PCR system 2400 under the following conditions: an initial denaturation at 94°C for 5 minutes; 8 cycles of (1) 94°C for 45 seconds, (2) 55°C for 45 seconds, and (3) 72°C for 3 minutes; 21 cycles of (1) 94°C for 45 seconds, (2) 61°C for 45 seconds and (3) 72°C for 3 minutes; and a final extension of 72°C for 10 minutes. A portion of the PCR reaction was separated by gel electrophoresis using a 0.8 percent TAE gel. The gel electrophoresis revealed a 1.6 kb fragment. This fragment was (1) purified using a Qiagen Gel Extraction kit (Qiagen Inc., Valencia, CA), (2) treated with *Kpn* I and *Xba* I (New England BioLabs, Inc., Beverly, MA), and (3) subcloned into pUC18 that had also been treated with *Kpn* I and *Xba* I and gel purified. The resulting construct designated appUC18-SHDXS is depicted in Figure 18. The ligation was carried out with T4 DNA ligase at 16°C for 16 hours. Once ligated, 1 μL was used to electroporate *E. coli* ElectroMAX™ DH10B™ cells (Life Technologies, Inc., Rockville, MD). The electroporated cells were plated on LB-Amp plates (Amp concentration = 100 μg/mL). From these plates, eight individual colonies were chosen at random. The plasmid was isolated from each colony using a QiaPrep Spin Miniprep kit (Qiagen Inc.,

Valencia, CA). The extracted plasmid DNA was examined for the presence of the 1.6 kb fragment by digesting individual aliquots with one of three different restriction enzymes: *EcoR* I, *BamH* I, and *Nar* I. If the plasmids contained the correct 1.6 kb fragment, the *EcoR* I digest reaction would result in two fragments (0.77 and 4.13 kb), the *BamH* I digest reaction would result in one fragment (4.8 kb), and the *Nar* I digest reaction would result in two fragments (1.9 and 2.9 kb). After treating with the restriction enzymes, the digest reactions were separated by gel electrophoresis using a 0.8 percent TAE agarose gel. All 8 clones yielded digestion fragments consistent with a clone of 1.6 kb.

Example 2 – Introducing nucleic acid that encodes a polypeptide having DXS activity into cells

The nucleic acid molecule that encodes a polypeptide having DXS activity and was obtained as described in Example 1 is introduced into cells as follows. First, a construct is made to contain the nucleic acid molecule such that the encoded polypeptide having DXS activity is expressed in a desired host cell. When using prokaryotic cells, a construct functional in prokaryotic cells is used. When using eukaryotic cells, a construct functional in eukaryotic cells is used. Second, the construct is introduced into the desired host cell using appropriate methods. Once introduced, stable transformants are selected.

Example 3 – Cloning nucleic acid that encodes a *Rhodobacter sphaeroides* polypeptide having DDS activity

R. sphaeroides ATCC strain 17023 cells were grown in 550 R 8 A H media at 30°C and 100 rpm. The recipe for 550 R 8 A H media was provided by ATCC. Genomic DNA was isolated from *R. sphaeroides* cells as described in Example 1.

To isolate nucleic acid encoding an *R. sphaeroides* polypeptide having DDS activity, degenerate primers were designed and used as described in Example 1. Briefly, three degenerate forward primers (F4, F5, and F6) and four degenerate reverse primers (R4, R5, R6, and R7) were designed by comparing sequences of several clones that encode polypeptides have DDS, SDS, or ODS activity (Figure 16). The sequence of each degenerate primer was as follows:

F4: 5'-GGWGGHAARMGMMTKCGYCC-3' (SEQ ID NO:67)
F5: 5'-ACWYTGSTDCATGATGATGT-3' (SEQ ID NO:68)
F6: 5'-ACNYTNBTNCAYGAYGAYGT-3' (SEQ ID NO:69)
R4: 5'-TYRTCYACSACATCATCATG-3' (SEQ ID NO:70)
R5: 5'-TGHAVKACYTCACCYTCRGMAAT-3' (SEQ ID NO:71)
R6: 5'-TARTCNARDATRTCRTCDAT-3' (SEQ ID NO:72)
R7: 5'-TCRTCNCCNAYNKTYTTNCC-3' (SEQ ID NO:73)

These primers were used in all logical combinations in PCR using Taq polymerase (Roche Molecular Biochemicals, Indianapolis, IN) and 1 ng of genomic DNA per microliter of reaction mix. PCR was conducted using the touchdown PCR program as described in Example 1. Between about 4 μM and 8 μM of each PCR primer was used in each reaction, depending on the degree of degeneracy. After each PCR reaction was complete, a portion of each reaction was separated by gel electrophoresis using a 1.5 percent TAE agarose gel. The results from the gel electrophoresis yielded no fragments of the expected size. A second amplification reaction was then performed using each sample from the first round of PCR. Briefly, one μL of reaction mixture from each first round of PCR was used in a 50 μL amplification reaction using the same primer pairs and thermocycling parameters used in the first round of PCR. A portion of each of the second round PCR reactions was separated by gel elecrophoresis using a 1.5 percent TAE agarose gel. The combination of degenerate primers F6 and R5 produced a fragment of 209 bp (referred to as the F6R5 fragment). The F6R5 fragment was isolated from an agarose gel and purified using the Qiagen Gel Extraction procedure (Qiagen Inc., Valencia, CA). An aliquot of the purified fragment was ligated to pCRII-TOPO, and the product of the ligation reaction was inserted into TOP10 *E. coli* cells using a TOPO cloning procedure (Invitrogen, Carlsbad, CA). The products of the individual insertion reactions were plated onto LB media containing 100 μg/mL Amp and 50 μg/mL Xgal. Single white colonies that grew on the LB-Amp-Xgal plates were re-plated onto fresh LB-Amp plates and screened in a PCR reaction using the F6 and R5 primers to confirm the presence of the desired insert. Plasmid DNAs were obtained from several colonies using a QiaPrep Spin Miniprep kit (Qiagen, Inc). The obtained plasmid DNAs were

quantified and sequenced with the M13 forward and reverse primers. Sequence analysis revealed that the F6R5 fragment contained sequences that aligned with sequences from other nucleic acid molecules that encode polypeptides having polyprenyl diphosphate synthase activity.

Genome walking was performed to obtain a complete coding sequence for the *R. sphaeroides* DDS polypeptide using procedures similar to those described in Example 1. Briefly, primers were designed based on the sequence of the F6R5 fragment for walking in both the upstream and downstream directions. These primers had the following sequences:

GSP3F: 5'-TGGAAGCTGCGGGCGAAGAGATAGTC-3' (SEQ ID NO:74)
GSP4F: 5'-CCCACCAGCACCGAGGATTTGTTGTC-3' (SEQ ID NO:75)
GSP3R: 5'-GAACCTGCTGTGGGACAACAAATCCTC-3' (SEQ ID NO:76)
GSP4R: 5'-TCGGTGCTGGTGGGCGACTATCTCTTC-3' (SEQ ID NO:77)

The GSP3F and GSP4F primers are primers that face downstream of the DDS polypeptide start codon, while the GSP3R and GSP4R primers are primers that face in the opposite direction. In addition, the GSP4F and GSP4R primers are nested inside the GSP3F and GSP3R primers.

The *Pvu* II, *Fsp* I, and *Stu* I libraries with the GSP3F and GSP4F primers and all four libraries with the GSP3R and GSP4R primers resulted in the production of amplified fragments. A 750 bp fragment from the *Pvu* I library, a 500 bp fragment from the *Fsp* I library, a 1.4 kb fragment from the *Stu* I library, and a 0.9 kb fragment from the *Sma* I library were all subcloned and sequenced. Sequence analysis indicated that each subcloned fragment contained a sequence that overlapped with the sequence of the F6R5 fragment and was similar to other nucleic acid sequences that encode polypeptides having polyprenyl diphosphate synthase activity. The full-length clone containing coding and non-coding sequence was 1990 bp in length (Figure 7). The open reading frame was 1002 bp in length (Figure 8), which encoded a polypeptide with 333 amino acid residues (Figure 9).

The coding sequence of the DDS polypeptide from *R. sphaeroides* was amplified by PCR using *R. sphaeroides* genomic DNA as template. PCR primers were designed based on the sequences obtained as described above. The sequences of the primers were as follows.

RDS18F: 5'-ACTAGAATTCCGCAACAGTTCCTTCATGTC-3' (SEQ ID NO:78)
RDS18R: 5'-ATAGAAGCTTACTTGCGGTCGGACTGATAG-3' (SEQ ID NO:79)

These primers were designed to introduce an *EcoR* I restriction site at the beginning of the amplified fragment and a *Hind* III restriction site at the end of the amplified fragment. The sequence of each restriction site is underlined. The PCR reaction mix contained the following: 100 ng genomic DNA, 2 μL of each primer (RDS18F and RDS18R, each at 50 μM), 10 μL 10X *Pfu* Plus buffer, 5 μL DMSO, 8 μL dNTPs (10 mM each) and 5 units *Pfu* polymerase in a final volume of 100 μL. Each PCR reaction was performed in a Perkin Elmer Geneamp PCR system 2400 under the following conditions: an initial denaturation at 94°C for 5 minutes; 8 cycles of (1) 94°C for 45 seconds, (2) 55°C for 45 seconds, and (3) 72°C for 3 minutes; 21 Cycles of (1) 94°C for 45 seconds, (2) 61°C for 45 seconds, and (3) 72°C for 3 minutes; and a final extension of 72°C for 10 minutes. After completing the PCR reactions, each PCR reaction was separated by gel electrophoresis using a 0.8 percent TAE agarose gel. The gel electrophoresis revealed a 1.6 kb fragment. This fragment was (1) purified from the agarose gel using a Qiagen Gel Extraction kit, (2) digested with *EcoR* I and *Hind* III (New England BioLabs, Beverly, MA), and (3) ligated to pUC18 that had also been digested with *EcoR* I and *Hind* III and gel purified. The resulting construct designated appUC18-RSdds is depicted in Figure 19. The ligation was carried out with T4 DNA ligase at 16°C for 16 hours. Once ligated, one μL of the ligation reaction was used to electroporate *E. coli* ElectroMAX™ DH10B™ cells (Life Technologies, Inc., Rockville, MD). The electroporated cells were plated onto LB-Amp plates (Amp concentration was 100 μg/mL). From these LB-Amp plates, eight individual colonies were selected at random, and the plasmids within these colonies were purified using a Qiaprep Spin Miniprep kit. These purified plasmids were evaluated for the presence of inserts by restriction enzyme analysis. If the plasmids contained the correct

1.6 kb fragment, then an *EcoR* I and *Hind* III digest reaction would result in two fragments (2.6 and 1.6 kb), and a *BamH* I digest reaction would result in one fragment (4.2 kb). After treating with the restriction enzymes, the digest reactions were separated by gel electrophoresis using a 0.8 percent TAE agarose gel. Of the eight clones tested, four contained the desired 1.6 kb fragment.

Example 4 – Cloning nucleic acid that encodes a *Sphingomonas trueperi* polypeptide having DDS activity

S. trueperi cells were grown as described in Example 1. In addition, genomic DNA was isolated from *S. trueperi* cells as described in Example 1.

To isolate nucleic acid encoding a polypeptide having DDS activity from *S. trueperi*, a strategy similar to that described in Example 3 was employed. In this case, four degenerate forward primers (SF1, SF2, SF3, and SF4) and four degenerate reverse primers (SR1, SR2, SR3, and SR4) were designed comparing sequences of several clones that encode polypeptides having polyprenyl diphosphate synthase activity (Figure 17). Codon usage tables from twelve *Sphingomonas* species were used to develop an average preferred codon table that was used in primer design. The sequence of each degenerate primer was as follows:

SF1: 5'-CTSSTSCAYGAYGAYGTSGTSGA-3' (SEQ ID NO:80)
SF2: 5'-GTSGMVGSSGGSGGSAARC-3' (SEQ ID NO:81)
SF3: 5'-CTSMTSCAYGAYGAYGTS-3' (SEQ ID NO:82)
SF4: 5'-DSSRTBCTSGTSGGSGAYTT-3' (SEQ ID NO:83)
SR1: 5'-VAKRAARTCSCCSACSAGSAC-3' (SEQ ID NO:84)
SR2: 5'-SACYTCSCCYTCSGCRAT-3' (SEQ ID NO:85)
SR3: 5'-RTCRTCSCCVAYVKTYTTSCC-3' (SEQ ID NO:86)
SR4: 5'-SGGSAGSGTVRBYTTSCCYTC-3' (SEQ ID NO:87)

The primers were used in all logical combinations in PCR using Taq polymerase (Roche Molecular Biochemicals, Indianapolis, IN) and 1 ng of genomic DNA per microliter of reaction mix. PCR was conducted using the touchdown PCR program as

described in Example 1. Between about 4μM and 20 μM of each PCR primer was used in each reaction depending on the degree of degeneracy. After each PCR reaction was complete, a portion of each reaction was separated by gel electrophoresis using a 1.5 percent TAE agarose gel. Each PCR reaction produced several amplified fragments of the expected sizes based on the coding sequences of other polyprenyl diphosphate synthase polypeptides. These fragments were isolated from TAE agarose gels and purified using the Qiagen Gel Extraction procedure (Qiagen Inc., Valencia, CA). An aliquot of each purified fragment was ligated into pCRII-TOPO. The ligated plasmids were then inserted into TOP10 *E. coli* cells using a TOPO cloning procedure (Invitrogen, Carlsbad, CA). The products of each of the individual insertion reactions were plated on LB-Amp-Xgal plates as described in Examples 1 and 3. Single white colonies that grew on the LB-Amp-Xgal plates were re-plated onto fresh LB-Amp-Xgal plates and screened in a PCR reaction using the initial degenerate primers to confirm the presence of the desired insert. Plasmid DNAs having the desired insert were obtained from multiple colonies using a QiaPrep Spin Miniprep Kit (Qiagen, Inc). The obtained plasmid DNAs were then quantified and sequenced using the M13 forward and reverse primers. Sequence analysis revealed that a 201 bp fragment produced using the SF1 and SR2 degenerate primers, a 476 bp fragment produced using the SF1 and SR4 primers, and a 206 bp fragment produced using the SF3 and SR2 primers contained sequences similar to the coding sequences of other polyprenyl diphosphate synthases.

Genome walking was performed to obtain a complete coding sequence for the *S. trueperi* DDS polypeptide using procedures similar to those described in Example 1. Briefly, primers were designed based on the sequences of the obtained fragments. These primers had the following sequences:

GSP5F: 5'-GTGCTGGTCGGCGACTTCCTGTTCAG-3' (SEQ ID NO:88)
GSP6F: 5'-ATCGACCTGTCCGAGGATCGCTATCTC-3' (SEQ ID NO:89)
GSP5R: 5'-TCGAACGAGCGGCTGAACAGGAAGTC-3' (SEQ ID NO:90)
GSP6R: 5'-TGGCGGGATTGCCCCAGATGATGTTG-3' (SEQ ID NO:91)

The GSP5F and GSP6F primers are primers that face downstream of the DDS start codon, while the GSP5R and GSP6R primers are primers that face in the opposite direction. In addition, the GSP6F and GSP6R primers are nested inside the GSP5F and GSP5R primers.

Genome walking was conducted as described in Example 3 with the exception that the 36 cycles had 3 minute incubations at 66°C instead of 67°C and the final extension was performed at 66°C instead of 67°C for both the first and second rounds of PCR. Portions of the PCR reactions from each round were separated by gel electrophoresis using a 1.5 percent TAE agarose gel. PCR on the *Fsp* I and *Stu* I libraries with the forward primers and of all four libraries with the reverse primers resulted in the production of an amplified fragment. A 1.4 kb fragment from the *Fsp* I library, a 1.1 kb fragment from the *Stu* I library (forward primer), a 2.0 kb fragment from the *Pvu* II library (forward primer), and a 3.0 kb fragment from the *Stu* I library (reverse primer) were gel purified, cloned using the TOPO cloning procedure, and sequenced as described in Examples 1 and 3. The sequencing analysis revealed that these fragments contained sequences that overlapped with the sequence of the initially obtained fragments and were similar to the coding sequences of other polyprenyl diphosphate synthases. The full-length clone containing coding and non-coding sequence was 1833 bp in length (Figure 10). The open reading frame was 1014 bp in length (Figure 11), which encoded a polypeptide with 337 amino acid residues (Figure 12).

The coding sequence of the DDS polypeptide from *S. trueperi* was amplified by PCR using *S. trueperi* genomic DNA as template. PCR primers were designed based on the sequences obtained as described above. The sequences of the primers were as follows.

SHDDSF: 5'-ATTAGGTACCATCAGATAATCGTCGCTCAA-3' (SEQ ID NO:92)
SHDDSR: 5'-TATAGGATCCGACATGGACGAGGAAGACGC-3' (SEQ ID NO:93)

These primers were designed to introduce a *Kpn* I restriction site at the beginning of the amplified fragment and a *BamH* I restriction site at the end of amplified fragment. The sequence of each restriction site is underlined. The PCR reactions were performed as

described in Example 3 with the exception that primers SHDDSF and SHDDSR were used instead of RDS18F and RDS18R. Once the PCR was completed, the PCR reactions were separated by gel electrophoresis using a 0.8 percent TAE agarose gel. The gel electrophoresis revealed a 1.6 kb fragment. This 1.6 kb fragment was (1) purified using a Qiagen Gel Extraction kit, (2) digested with *Kpn* I and *BamH* I (New England BioLabs), and (3) ligated into pUC18 that had also been digested with *Kpn* I and *BamH* I and gel purified using methods similar to those described in Example 3. The resulting construct designated appUC18-SHDDS is depicted in Figure 20. This construct was used to transform cells as described in Example 3. The transformed cells were plated onto LB-Amp plates, and eight individual colonies were selected at random. Plasmid DNA was isolated from each colony using a QiaPrep Spin Miniprep kit. The extracted plasmid DNA was tested for the presence of the 1.6 kb fragment using three different restriction digests. If the plasmids contained the 1.6 kb fragment, then a *BamH* I and *Kpn* I digest would yield two fragments (2.68 and 1.62 kb), an *EcoR* I digest would yield two fragments (1.45 and 2.85 kb), and a *Ban* II digest would yield two fragments (0.48 and 3.8 kb). All eight plasmids tested yielded digestion fragments consistent with a plasmid containing the desired 1.6 kb fragment.

Example 5 – Measuring CoQ(10)

Harvested cells were suspended in water to have about 0.1 gm dry weight per mL. The suspension was subjected to a French-press, and the resulting in suspension was frozen in 1 mL aliquots until used.

To measure CoQ(10) in a sample, two aliquots were repeatedly thawed and refrozen 4-5 times. Once transferred to a 50 mL centrifuge tube, 1 mL of 5% sodium dodecyl sulfate was added to the thawed material. The material was then flushed with nitrogen. After vortexing for one minute, six mL of ethanol was added to the material, and the resulting mixture was vortexed for one minute. Then, 15 mL of hexane was added to the mixture. After vortexing for five minutes, the mixture was centrifuged at 3000 rpm for ten minutes. Once centrifuged, the hexane layer was removed to a conical flask and flushed with nitrogen. This hexane extraction was repeated two times. The three extracts were pooled into a single tube that was evaporated on a vacuum evaporator

until the residue was near dryness. The residue was dissolved in 2 mL of mobile phase by vortexing for 2-3 minutes. Once vortexed, the solution was transferred to a 5 mL volumetric flask. The tube that contained the residue was rinsed two additional times with 1 mL of mobile phase. Each time the rinse solution was transferred to the same 5 mL volumetric flask. After adjusting the total volume to 5 mL, the solution was mixed well and stored at -20°C until analyzed.

As a control, either water or a culture solution was spiked with standard CoQ(10), extracted as indicated above, and analyzed to determine the recovery of the spiked material. The CoQ(10) standard was a stock solution of CoQ(10), obtained from Sigma. The stock solution was made in HPLC grade ethanol at a concentration of 100 μg/mL, and then diluted to get CoQ(10) solutions ranging from 100 μg/mL to 1 μg/mL.

HPLC analysis was performed with the following parameters. The mobile phase was ethanol:methanol (7:3) or methanol:isopropylether (9:1). The flow rate was 0.75 mL/min. The column was Waters Nova-Pak C18 (3.9 x150 mm; 4Um). The detector was a PDA set from 200-300 nm with the resolution at 1.2 nm and the maximum absorbance at 275 nm. The run time was 15 minutes, and the injection volume was 50 μL. To calculate the amount of CoQ(10) present, 50 μL of each sample was injected, and the results compared to those obtained using the calibration curve. From these data points, the concentration per gm dry weight was calculated.

Example 6 – Introducing nucleic acid that encodes a polypeptide having DDS activity into cells and measuring isoprenoid levels

The following procedures were followed individually for the *R. sphaeroides* and *S. trueperi* nucleic acid isolated as described in Examples 3 and 4, respectively.

Plasmid DNA encoding the polypeptide having DDS activity was electroporated into wild type *E. coli* strain MG1655. The electroporated cells were plated onto LB-Amp plates. A single individual bacterial colony was picked for each DDS coding sequence, and each colony was grown overnight in 2 mL of LB-Amp at 37°C with 200 rpm shaking. About 0.75 mL of these overnight cultures were used to inoculate flasks containing 75 mL LB-Amp medium (Amp concentration was 100 μg/mL). These second cultures were grown at 37°C at 200 rpm for 30 hours. Additional Amp (to a final concentration of 50

μg of fresh Amp per mL) was added to each flask after 12 hours of growth. After 30 hours, the bacteria were collected by centrifugation at 8,000 g for 10 minutes. The resulting bacterial cell pellets were washed by adding 20 mL of 10 mM Tris-HCL buffer (pH 8.0), resuspending the cells, and re-centrifuging as before. Each cell pellet was then resuspended in 10 mL of water. About 0.5 mL of each extract was used for dry mass analysis and the remaining cell suspensions (about 9.5 mL) were frozen at -20°C overnight.

The 9.5 mL cell suspensions were used as follows. First, the cells were thawed on ice and lysed by passing the cell suspensions through a French press three times (14,000 psi pressure). The resulting cell extracts were frozen at –20°C in 1 mL aliquots and maintained on ice prior to analysis.

High pressure liquid chromatography was performed using Waters' 2690 Alliance integrated system (Waters Corporation, Milford, Mass). Prior to analysis, all samples and standards were dissolved in HPLC-grade ethanol, loaded into the built-in auto-sampler, and kept at 5°-10°C in the dark. The separation was carried out using an isocratic elution program of 70:30 ethanol/methanol (v/v) at a flow rate of 1.0 mL/min. The column was a Waters Nova-Pak C18, 3.9-150 mm equipped with a guard column of the same stationary phase. The injection volume was typically 10-25 μL. Total run time was ten minutes.

Under these conditions, retention times were 3.1 and 4.9 minutes for CoQ(8) and CoQ(10), respectively. For quantification purposes, a four-point external calibration curve was calculated using freshly prepared CoQ(10) standards. Calibration levels were 1.0, 4.0, 10.0 and 100.0 μg/mL (ppm). Each standard was injected in triplicate, and the resulting calibration plot was linearly fitted with observed r^2's of >0.999.

For UV and MS detection, a photodiode array (PDA, Model UV6000LP, ThermoQuest Corp., San Jose, CA) and an ion trap mass analyzer (LCQ Classic, Finnigan/ThermoQuest Corp., San Jose, CA) were connected in series with the chromatograph and without splitting of the effluent. The PDA was operated in scanning mode from 220-300 nm. Effluent from the PDA was introduced into the mass analyzer via atmospheric-pressure chemical ionization (APCI) using the following parameters: capillary temperature, 150°C; capillary voltage, 3kV; vaporizer temperature, 400°C; sheath gas (N_2) flow, 80 arbitrary units; auxiliary gas (N_2) flow, 5 arbitrary units; and

corona discharge needle, 5mA/6kV. Positive-ion detection was performed in full scan (250-1000 m/z), 2 mscans, 500 ms ion injection time.

Under these conditions, CoQ(8) yielded a mass spectrum with a base peak at 727.5 m/z, corresponding to the protonated 'molecular ion' as well as several satellite ions from ethanol and/or methanol adducts (Figures 23 and 24). Similarly, CoQ(10) yielded a mass spectrum with a base peak at 863.6 m/z corresponding to its protonated 'molecular ion' (Figure 25). Several ethanol and/or methanol satellite adducts were observed as well. Both CoQ(8) and CoQ(10) yielded UV spectra with maxima at 274 nm.

Two samples were analyzed: MG1655 PUC18 and MG1655 PUC18-DDS. MG1655 PUC18 is *E. coli* strain MG1655 transfected with the PUC18 vector only. MG1655 PUC18-DDS is *E. coli* strain MG1655 transfected with the PUC18 vector containing nucleic acid that encodes a *R. sphaeroides* polypeptide having DDS activity. The MG1655 PUC18 specimen contained only CoQ(8) (retention time 3.08 min, Figure 21) as confirmed by its mass spectrum (Figure 23), with a base peak at 727.4 m/z and a UV spectrum with a maximum at 274 nm. The MG1655 PUC18-DDS specimen, however, contained CoQ(8) and CoQ(10) (Figure 22), both of which were confirmed by matching mass spectra (Figures 24 and 25) and UV maxima.

Example 7 – Cloning nucleic acid that encodes a *Sphingomonas trueperi* polypeptide having DXR activity

Sphingomonas trueperi ATCC 12417 cultures (100-200 mL) were grown in nutrient broth at 30°C and 250 rpm for 2-3 days. The cells then were pelleted and washed with a 10 mM Tris:1.0 mM EDTA solution. The pellets were resuspended in 5 mL of GTE buffer (50 mM glucose, 25 mM Tris HCl (pH 8.0), 10 mM EDTA (pH 8.0)) per 100 mL of culture. Lysozyme and Proteinase K were added to a 1 mg/mL concentration and mutanolysin was added to 5.5 μg/mL. After a 1.5 hour incubation at 37°C, SDS was added to a final concentration of 1%, and the concentration of Proteinase K was brought to 2 mg/mL. After incubation at 50°C for one hour, an equal volume of GTE buffer was added, and NaCl was added to a 0.15 M concentration. The mixture was extracted with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) and centrifuged at

10,000 rpm for 10 minutes. The supernatant was removed to a clean tube, extracted with an equal volume of chloroform, and centrifuged at 5,000 rpm for 10 minutes. The supernatant was treated with RNAse and precipitated with 2.5 volumes of ethanol. The spooled DNA was washed with 70% ethanol, air dried, and resuspended in 10 mM Tris (pH 8.5). After resuspending, the resuspended DNA was further cleaned by re-extraction with phenol:chloroform:isoamyl alcohol and chloroform, and reprecipation with 1/10 volume 7.4 M NH_4OAc and 2.5 volumes ethanol.

A conserved region of the 1-deoxy-D-xylulose 5-phosphate reductoisomerase (dxr) gene was cloned by PCR. Five degenerate forward and five degenerate reverse PCR primers were designed from conserved protein regions that were revealed by aligning known dxr genes (Figure 27). The degenerate sequences were designed from the conserved regions using the universal codon table. The primers were used in all logical combinations in PCR using Taq polymerase (Roche Molecular Biochemicals, Indianapolis, IN) and 1 ng of genomic DNA/μL reaction mix. PCR was conducted using a touchdown PCR program with 4 cycles at an annealing temperature of 59°C, 4 cycles at 57°C, 4 cycles at 55°C, and 24 cycles at 53°C. Each cycle used an initial 30 second denaturing step at 94°C and a 1.75 minute extension at 72°C, and the program had an initial denaturing step for 2 minutes at 94°C and final extension of 5 minutes at 72°C. The amounts of PCR primer used in the reaction were increased 3-12 fold above typical PCR amounts depending on the amount of degeneracy in the 3' end of the primer. In addition, separate PCR reactions containing each individual primer were made to identify PCR products resulting from single degenerate primers. Fifteen μL of each PCR product was separated on a 1.5% TAE (Tris-acetate-EDTA)-agarose gel. Degenerate primers F2 (5'-CCSGTSGAYWSSGARCAYAACGCS-3' (SEQ ID NO:132)) and R7 (5'-ATGATGAACAAGGGSCTSGAR-3' (SEQ ID NO:133)) produced a band of about 250 bp, which was the expected size based on dxr genes from other species. This band was not present in the individual F2 and R7 primer control reactions. Degenerate primers F3 (5'-CATCCVAACTGGWMVATGGG-3' (SEQ ID NO:134)) and R2 (5'-ATYGGYRWWCKCATATCMGG-3' (SEQ ID NO:135)) produced a band of about 200 bp, which also was the expected size. The F2-R7 and F3-R2 fragments were isolated and purified using a QIAquick Gel Extraction Kit (Qiagen Inc., Valencia, CA). Three μL of

the purified band was ligated into pCR®II-TOPO vector, which was then transformed by a heat-shock method into TOP10 *E. coli* cells using a TOPO cloning procedure (Invitrogen, Carlsbad, CA). Transformations were plated on LB media containing 100 μg/mL of ampicillin and 50 μg/mL of 5-Bromo-4-Chloro-3-Indolyl-B-D-Galactopyranoside (X-gal). Individual, white colonies were resuspended in about 20 μL of 10 mM Tris and heated for 10 minutes at 95°C to break open the bacterial cells. To screen individual colonies, 2 μL of the heated cells was used in a 25 μL PCR reaction as described above using the appropriate degenerate primers. Plasmid DNA was obtained with a QIAprep Spin Miniprep Kit (Qiagen, Inc) from cultures of colonies having the desired insert and used for DNA sequencing with M13R and M13F primers. Sequence analysis revealed that the F2-R7 and F3-R2 fragments overlapped and were homologous to known dxr genes.

Genome walking was performed to obtain the complete coding sequence as follows. The overlapping of the F2-R7 and F3-R2 fragments resulted in a sequence 358 bp in length. The following four primers for conducting genome walking in both upstream and downstream directions were designed using the portion of this sequence that was internal to the degenerate primers:

GSP1F 5'-CGAATGGACGACGGATTGGCGATGGAC-3' (SEQ ID NO:136)
GSP2F 5'-TCAGTTCGAGCCCCTTGTTCATCATCGTC-3' (SEQ ID NO:137)
GSP1R 5'-CGAACTGATCGAAGCCTTCCACCTGTTC-3' (SEQ ID NO:138)
GSP2R 5'-GGTCCATCGCCAATCCGTCGTCCATTC-3' (SEQ ID NO:139)

The GSP1F and GSP2F primers faced upstream, the GSP1R and GSP2R primers faced downstream, and the GSP2F and GSP2R primers were nested inside the GSP1F and GSP1R primers. Genome walking was conducted according to the manual for CLONTECH's Universal Genome Walking Kit (CLONTECH Laboratories, Inc., Palo Alto, CA) with the exception that the enzymes FspI and SmaI were used in place of DraI and EcoRV. The DraI and EcoRV enzymes were replaced because they cut *S. trueperi* genomic DNA too infrequently to give fragment lengths amenable to PCR. The PCR mixture contained 5% DMSO. First round PCR was conducted in a Perkin Elmer 9700

Thermocycler with 7 cycles consisting of 2 seconds at 94°C and 3 minutes at 72°C, and 36 cycles consisting of 2 seconds at 94°C and 3 minutes at 66°C, with a final extension at 66°C for 4 minutes. Second round PCR used 5 cycles consisting of 2 seconds at 94°C and 3 minutes at 72°C, and 26 cycles consisting of 2 seconds at 94°C and 3 minutes at 66°C, with a final extension at 66°C for 4 minutes. Nine μL of the first round product and seven μL of the second round product were separated on a 1.5% TAE-agarose gel. A 1.3 Kb band was obtained from the second round product for the SmaI forward reaction, an 800 bp band for the StuI reverse reaction, and a 750 bp band for the PvuII reverse reaction. These fragments were gel purified, cloned, and sequenced. Internal primers were used to amplify and obtain additional sequence of the gene. Sequence analysis revealed that the sequence derived from genome walking overlapped with the original fragments and contained an entire coding sequence homologous to known dxr genes. The full-length clone containing coding and non-coding sequence was 2017 bp in length (Figure 28). The open reading frame starting with the first GTG site was 1161 bp in length (Figure 29), which encoded a polypeptide with 386 amino acid residues (Figure 30).

Example 8 – Making recombinant microorganisms

Rhodobacter sphaeroides (ATCC 35053) was routinely maintained on Luria Bretain (Miller) agar (Fisher scientific) plates. When needed, *R. sphaeroides* was cultured as follows. A 5 mL culture was grown in a 15 mL culture tube at 30°C in Innova 4230 Incubator, Shaker (New Brunswick Scientific, Edison, NJ) with a shaking speed of 250 rpm. Each 5 mL culture was started by inoculating liquid media (Sistrom media supplemented with 20% LB) with a single colony. The liquid media contained the following ingredients per liter: 2.72 g KH_2PO_4, 0.5 g $(NH_4)_2SO_4$, 0.5 g NaCl, 0.2 g EDTA disodium salt, 0.3 g $MgSO_4 \cdot 7H_2O$, 0.033 g $CaCl_2 \cdot 2H_2O$, 0.2 mg $FeSO_4 \cdot 7H_2O$, 0.02 mL $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (1% solution), 1 mL Trace element solution, 0.2 mL Vitamin solution, 5 g Luria Bretain Broth Mix, and 8 mL Glucose (50%). The Trace element solution contained the following ingredients per liter: 1.765 g EDTA disodium salt, 10.95 g $ZnSO_4 \cdot 7H_2O$, 5 g $FeSO_4 \cdot 7H_2O$, 1.54 g $MnSO_4 \cdot H_2O$, 0.392 g $CuSO_4 \cdot 5H_2O$, 0.284 g $Co(NO_3)_2 \cdot 6H_2O$, and 0.114 g H_3BO_3. The Vitamin solution contained the following

ingredients per liter: 10 g Nicotinic acid, 5 g Thiamine HCl, and 0.01 g Biotin. The vitamins and glucose were added after the media cooled to room temperature after autoclaving. When necessary, the media was supplemented with one or more of the following antibiotics: Kanamycin (25 μg/mL; final concentration), Spectinomycin (25 μg/mL; final concentration), and/or Streptomycin (25 μg/mL; final concentration).

Electrocompetent *R. sphaeroides* cells

Electrocompetent *R. sphaeroides* cells were made as follows. A 5 mL culture of *R. sphaeroides* was grown overnight at 30°C in Sistrom's media supplemented with 20% LB. This culture was diluted 1/100 in 300 mL of the same media and grown to an OD_{660} of 0.5-0.8. The cells were chilled on ice for 10 minutes and then centrifuged for 6 minutes at 7,500 g. The supernatant was discarded, and the cell pellet was resuspended in ice-cold 10% glycerol at half of the original volume. The cells were pelleted by centrifugation for 6 minutes at 7,500 g. The supernatant was again discarded, and cells resuspended in ice-cold 10% glycerol at one quarter of the original volume. The last centrifugation and resuspension steps were repeated, followed by centrifugation for 6 minutes at 7,500 g. The supernatant was decanted, and the cells resuspended in the small volume of glycerol that did not drain out. Additional ice-cold 10% glycerol was added to resuspend the cells, if necessary. Forty μL of the resuspended cells was used in a test electroporation to determine if the cells needed to be concentrated by centrifugation or diluted with 10% ice-cold glycerol. Time constants of 8.5-9.0 milliseconds resulted in good transformation efficiencies. If cells were too dilute, the time constant was greater than 9.0 and transformation efficiencies were low. If cells were too concentrated, the electroporation would spark. Once an acceptable time constant was achieved, cells were aliquoted into cold microfuge tubes and stored at -80°C. All water used for media and glycerol was 18.2 Mohm-cm or higher.

Electrocompetent *R. sphaeroides* cells were electroporated as follows. One μL of plasmid DNA was gently mixed into 40 μL of *R. sphaeroides* electrocompetent cells, which were then transferred to an electroporation cuvette with a 0.2 cm electrode gap. Electroporations were conducted using a Biorad Gene Pulser II (Biorad, Hercules, CA) with settings at 2.5 kV of energy, 400 ohms of resistance, and 25 μF of capacitance. Cells

were recovered in 400 μL SOC media at 30°C for 6-16 hours. The cells were then plated (200 μL per plate) on the appropriate selective media. Transformation efficiencies averaged about 2,000 transformants/μg of DNA.

Electrocompetent *E. coli* cells

Electrocompetent *E. coli* strain S17-1 cells were made as follows. A 5 mL culture of *E. coli* strain S17-1 was grown overnight at 30°C in LB media supplemented with 25 μg/mL of streptomycin and 25 μg/mL of spectinomycin. This culture was diluted 1/100 in 300 mL of the same media and grown to an OD_{660} of 0.5-0.8. The cells were chilled on ice for 10 minutes and then centrifuged for 6 minutes at 7,500 g. The supernatant was discarded, and the cell pellet was resuspended in ice-cold 10% glycerol at half of the original volume. The cells were pelleted by centrifugation for 6 minutes at 7,500 g. The supernatant was again discarded, and the cells were resuspended in ice-cold 10% glycerol at one quarter of the original volume. The last centrifugation and resuspension steps were repeated, followed by centrifugation for 6 minutes at 7,500 g. The supernatant was decanted, and the cells resuspended in the small volume of glycerol that did not drain out. Additional ice-cold 10% glycerol was added to resuspend the cells, if necessary. Cells were aliquoted into cold microfuge tubes and stored at -80°C.

Electrocompetent *E. coli* strain S17-1 cells were electroporated as follows. Forty μL of competent cells was used per electroporation. Electroporation was conducted using a Biorad Gene Pulser II and a standard *E. coli* protocol: 2.5 kV of energy, 200 ohms of resistance, and 25 μF of capacitance. Electroporated cells were recovered in 250-1000 μL of SOC media for one hour, and 10-200 μL of culture was plated per plate of selective media. Transformation efficiencies averaged about 1.5×10^4 transformants/μg of DNA.

Constructs

Various clones were overexpressed in *R. sphaeroides* using the broad-host-range vector pBBR1MCS2 (Kovach *et al.*, *Gene*, 166:175-176 (1995)) that was engineered to have either an *R. sphaeroides* rrnB promoter, an *R. sphaeroides* glnB promoter, or a tet promoter. The pBBR1MCS2 vector is mobilizable and relatively small (5,144 bp), replicates in *R. sphaeroides*, has a multiple cloning site with lacZα color selection, and

carries a kanamycin resistance gene. All restriction enzymes and T4 DNA ligase were obtained from New England Biolabs (Beverly, MA) unless otherwise indicated. All plasmid DNA preparations were done using QIAprep Spin Miniprep Kits or Qiagen Maxi Prep Kits, and all gel purifications were done using QIAquick Gel Extraction Kits (Qiagen, Valencia, CA).

pMCS2rrnBP

The vector designated pMCS2rrnBP, which contains an *R. sphaeroides* rrnB promoter, was constructed by inserting a copy of the *R. sphaeroides* rrnB promoter (rrnBP) into the pBBR1MCS2 vector. The rrnB promoter was isolated from the pTEX124 vector (obtained from S. Kaplan) by digestion with the restriction enzyme BamHI, which releases the promoter as a 363 bp fragment. Alternatively, the rrnB promoter can be obtained by PCR amplifying it from *R. sphaeroides* genomic DNA using primers based on published rrnB sequence (GenBank® accession number X53854). This fragment was gel purified from a 2% Tris-acetate-EDTA (TAE) agarose gel. The pBBR1MCS2 vector was also digested with BamHI, and the enzyme heat inactivated at 80°C for 20 minutes. The digested vector was then dephosphorylated with shrimp alkaline phosphatase (Roche Moelcular Biochemicals, Indianapolis, IN) and gel purified from a 1% TAE-agarose gel. The prepared vector and the rrnBP fragment were ligated using T4 DNA ligase at 16°C for 16 hours. One μL of ligation reaction was used to electroporate 40 μL of *E. coli* Electromax™ DH10B™ cells (Life Technologies, Inc., Rockville, MD). Electroporated cells were plated on LB media containing 25 μg/mL of kanamycin (LBK). Plasmid DNA was isolated from cultures of single colonies and was digested with HindIII restriction enzyme to confirm the presence of a single insertion of the rrnB promoter. The sequence of the rrnBP inserts for these colonies was also confirmed by DNA sequencing.

pMCS2glnBP

The vector designated pMCS2glnBP, which contains an *R. sphaeroides* glnB promoter, was constructed by inserting a copy of the *R. sphaeroides* glnB promoter (glnBP) into the pBBR1MCS2 vector. The glnB promoter was PCR amplified from

genomic DNA obtained from *R. sphaeroides* strain 35053. The following primers were designed based on sequence information obtained from GenBank® accession number X71659:

glnBF 5'-ATTATCTAGAATCCGCCCCGCCTCCACCTC-3' (SEQ ID NO:140)
glnBR 5'-GATGGATCCTGGGTAGGGTCGCTGCTGTCC-3' (SEQ ID NO:141)

The primers introduced an XbaI restriction site at the 5' end and a BamHI restriction site at the 3' end. The following reaction mix and PCR program was used to amplify the promoter region of the glnB gene.

Reaction Mix	
Pfu 10X buffer	10 µL
DMSO	5 µL
dNTP mix (10 mM)	4 µL
glnBF (50 µM)	2 µL
glnBP (50 µM)	2 µL
Genomic DNA (50ng/µL)	2 µL
Pfu enzyme (2.5 U/µL)	2 µL
DI water	73 µL
Total:	100 µL

PCR program	
94°C	2 minutes
7 cycles of:	
94°C	30 seconds
61°C	45 seconds
72°C	3 minutes
25 cycles of:	
94°C	30 seconds
66°C	45 seconds
72°C	3 minutes
72°C	7 minutes
4°C	Until used further

The PCR product was separated on a 1.2% TAE-agarose gel. An about 500 bp fragment was excised and gel purified. The isolated DNA was restricted with XbaI and BamHI, and the resulting digested DNA column purified using a Qiagen gel isolation kit. Three µg of pBBR1MCS2 plasmid DNA was digested with BamHI and XbaI. The digestion was inactivated at 80°C for 20 minutes. The digested vector was then dephosphorylated with shrimp alkaline phosphatase and gel purified on a 1% TAE-agarose gel. Eighty-six ng of the prepared pBBR1MCS2 vector was ligated with 60 ng of the digested glnBP PCR product using T4 DNA ligase at 14°C for 14-16 hours. One µL of ligation reaction was used to electroporate 40 µL of *E. coli* Electromax™ DH10B™ cells. Electroporated cells were plated on LB media containing 25 µg/mL of kanamycin and 50 µg/mL of Xgal (LBKX). Eight individual, white colonies were selected, and their

plasmid DNA isolated using a QIAprep Spin Miniprep Kit. Plasmid DNA isolated from each colony was digested in separation reaction mixtures with PstI and a combination of EcoRI/XbaI. All eight clones had a restriction pattern that indicated the presence of the insert. The sequence of three clones was verified.

pMCS2tetP

The vector designated pMCS2tetP, which contains a tet promoter, was constructed by cloning the promoter for the tetracycline resistance determinants from transposon Tn1721 (Waters *et al.*, *Nucleic Acids Research*, 11(17):6089-6105 (1983)) into the pBBR1MCS2 vector. The tetA gene promoter (tetP) was amplified using plasmid pRK415 as template. The following primers were designed to introduce an XbaI restriction site at the beginning of the amplified fragment and a BamHI site at the end of the amplified fragment.

TETXBAF 5'-TTATCTAGAACCGTCTACGCCGACCTC-GTTCAAC-3' (SEQ ID NO:142)

TETBAMR 5'-TTAGGATCCCCTCCGCTGGTCCGATTG-AAC-3' (SEQ ID NO:143)

The PCR mix contained the following: 1X Native Plus Pfu buffer, 20 ng pRK415 plasmid DNA, 0.2 μM of each primer, 0.2 mM of each dNTP, 5% DMSO (v/v), and 10 units of native Pfu DNA polymerase in a final volume of 200 μL. The PCR reaction was performed in a Perkin Elmer Geneamp PCR System 2400 under the following conditions: an initial denaturation at 94°C for 1 minute; 8 cycles of 94°C for 30 seconds, 60°C for 45 seconds, and 72°C for 45 seconds; 24 cycles of 94°C for 30 seconds, 66°C for 45 seconds, and 72°C for 45 seconds; and a final extension for 7 minutes at 72°C. The amplification product was then separated by gel electrophoresis using a 2 %TAE-agarose gel. A 160 bp fragment was excised from the gel and purified. The purified fragment was digested simultaneously with XbaI and BamHI restriction enzymes, and purified with a QIAquick PCR Purification Kit. Three μg of pBBR1MCS2 plasmid DNA was digested with BamHI and XbaI, and the digest was inactivated at 80°C for 20 minutes. The

digested vector was then dephosphorylated with shrimp alkaline phosphatase and gel purified on a 1% TAE-agarose gel.

100 ng of the prepared pBBR1MCS2 vector was ligated with 36 ng of the digested tetP PCR product using T4 DNA ligase at 16°C for 16 hours. One μL of ligation reaction was used to electroporate 40 μL of *E. coli* Electromax™ DH5α™ cells. Electroporated cells were plated on LB media containing 25 μg/mL of kanamycin and 50 μg/mL of Xgal (LBKX). Individual, white colonies were resuspended in about 25 μL of 10 mM Tris, and 2 μL of the resuspension was plated on LBKX. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells. Two μL of the heated cells was used in a 25 μL PCR reaction using the following primers homologous to the vector and flanking the cloning site:

MCS2FS 5'-AGGCGATTAAGTTGGGTAAC-3' (SEQ ID NO:144)
MCS2RS 5'-GACCATGATTACGCCAAG-3' (SEQ ID NO:145)

The PCR mix contained the following: 1X Taq PCR buffer, 0.2 μM each primer, 0.2 mM each dNTP, and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 32 cycles of 94°C for 30 seconds, 55°C for 45 seconds, and 72°C for 1 minute; and a final extension for 7 minutes at 72°C. All colonies showed a single insertion event. Plasmid DNA was isolated from cultures of two individual colonies and sequenced to confirm the DNA sequence of the tet promoter in the construct.

pMCS2rrnBP/Stdxs

The nucleic acid encoding a *S. trueperi* polypeptide having DXS activity was cloned in the pMCS2rrnBP vector as follows. The *S. trueperi* dxs gene was amplified by PCR using primers homologous to sequence upstream and downstream of the gene. These primers, STDXSMCSF and STDXSMCSR, were designed to introduce a ClaI restriction site at the beginning of the amplified fragment and a KpnI site at the end of the amplified fragment.

STDXSMCSF 5'-GATAATCGATGTGTGACTGACCTGT-
CCAAC-3' (SEQ ID NO:146)
STDXSMCSR 5'-CTTAGGTACCATGTTGGAGATTCAA-
GGTGG-3'(SEQ ID NO:147)

The PCR mix contained the following: 1X Native Plus Pfu buffer, 200 ng *S. trueperi* genomic DNA, 0.2 μM of each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 10 units of native Pfu DNA polymerase (Stratagene, La Jolla, CA) in a final volume of 200 μL. The PCR reaction was performed in a Perkin Elmer Geneamp PCR System 2400 under the following conditions: an initial denaturation at 94°C for 1 minute; 8 cycles of 94°C for 30 seconds, 54°C for 45 seconds, and 72°C for 3.5 minutes; 27 cycles of 94°C for 30 seconds, 60°C for 45 seconds, and 72°C for 3.5 minutes; and a final extension for 7 minutes at 72°C. The amplification product was then separated by gel electrophoresis using a 1% TAE-agarose gel. A 2.2 Kb fragment was excised from the gel and purified. The purified fragment was digested with ClaI restriction enzyme, purified with a QIAquick PCR Purification Kit, digested with KpnI restriction enzyme, purified again with a QIAquick PCR Purification Kit, and quantified on a minigel.

Three μg of the pMCS2rrnBP vector was digested with the restriction enzyme ClaI, gel purified on a 1% TAE-agarose gel, digested with KpnI, purified with a QIAquick PCR Purification Kit, dephosphorylated with shrimp alkaline phosphatase, and purified again with a QIAquick PCR Purification Kit. 120 ng of the digested PCR product containing the *S. trueperi* dxs gene and the 50 ng of the prepared pMCS2rrnBP vector was ligated using T4 DNA ligase at 16°C for 16 hours. One μL of the ligation reaction was used to electroporate 40 μL of *E. coli* Electromax™ DH10B™ cells. The electroporated cells were plated onto media. Plasmid DNA was isolated from cultures of individual colonies and evaluated for the presence of the desired insert by restriction enzyme analysis with HindIII and SacI enzymes. The sequence of the Stdxs insert was confirmed by DNA sequencing. The resulting plasmid containing the Stdxs sequence under the control of the rrnB promotor was designated pMCS2rrnBP/Stdxs.

Purified pMCS2rrnBP/Stdxs plasmid DNA derived from a colony having the correct sequence was then electroporated into electrocompetent cells of *R. sphaeroides*

strain 35053. Plasmid DNA was isolated from cultures of individual *R. sphaeroides* colonies. Restriction patterns of plasmid preparations from *R. sphaeroides* are difficult to analyze due to the presence of multiple native plasmids in this species. To check the plasmid integrity in *R. sphaeroides*, one μL of the plasmid preparation from a transformed *R. sphaeroides* colony was used to re-tranform *E. coli* Electromax™ DH10B™ cells by electroporation. Electroporated cells were plated on LBK media. Plasmid DNA was isolated from cultures of individual colonies and evaluated using SacI and HindIII restriction digests.

pMCS2rrnBP/Stdxs2

A second pMCS2rrnBP plasmid containing the nucleic acid encoding a *S. trueperi* polypeptide having DXS activity was constructed. This construct was made using the following forward primer designed to introduce the ribosomal binding site (rbs) from the *R. sphaeroides* dxs1 gene along with a ClaI restriction site.

SXSCLAF2 5'-ACTATCGATGAAGGAAGAGCATGGCTGACCT-ACCCAAGAC-3' (SEQ ID NO:146)

S. trueperi genomic DNA was used as template in a PCR mixture using the primers SXSCLAF2 and STDXSMCSR. The PCR program and reaction mixture used were identical to those described for the pMCS2rrnBP/Stdxs construct. The PCR product was gel purified, digested with ClaI, purified with a QIAquick PCR Purification Kit, digested with restriction enzyme KpnI, and purified again with a QIAquick PCR Purification Kit. 150 ng of digested PCR product was ligated into 50 ng of the prepared pMCS2rrnBP vector using T4 DNA ligase at 16°C for 16 hours. One μL of the ligation reaction was transformed into *E. coli* Electromax™ DH10B™ cells, and the electroporated cells were plated onto LBK plates. Plasmid DNA was isolated from cultures of individual colonies and evaluated for the presence of the desired insert by restriction enzyme analysis with HindIII and SacI enzymes. The sequence of the dxs insert was confirmed by DNA sequencing. The resulting plasmid containing the Stdxs

sequence under the control of the rrnB promotor and having an *R. sphaeroides* ribosomal binding site was designated pMCS2rrnBP/Stdxs2.

A confirmed construct was electroporated into *R. sphaeroides* strain 35053, and the electroporated cells were plated onto LBK media. Individual colonies were resuspended in about 25 μL of 10 mM Tris, and 2 μL of the resuspension was plated on LBK media. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and two μL of the heated cells used in a 25 μL PCR reaction using the SXSCLAF2 and STDXSMCSR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 μM each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase (Roche) per reaction. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 54°C for 1 minute, and 72°C for 3.5 minutes; 24 cycles of 94°C for 30 seconds, 60°C 1 minute, and 72°C for 3.5 minutes; and a final extension for 7 minutes at 72°C.

pMCS2rrnBP/Rsdds

The nucleic acid encoding a *R. sphaeroides* polypeptide having DDS activity was cloned in the pMCS2rrnBP vector as follows. The *R. sphaeroides* dds gene was PCR amplified using the following primer pair:

RDS18F 5'-ACTAGAATTCCGCAACAGTTCCTTCATGTC-3' (SEQ ID NO:147)
RSDDSMCSR 5'-CTAGATCGATACTTGCGGTCGGACTGATAG-3' (SEQ ID NO:148)

The forward primer was located upstream of the start codon and introduced an EcoRI restriction site, while the reverse primer was located downstream of the stop codon and introduced a ClaI restriction site. Since the forward primer was located upstream, the *R. sphaeroides* dds maintained its native ribosomal binding site. The following reaction mix and PCR program were used to amplify the *R. sphaeroides* dds gene.

Reaction Mix	
Pfu 10X buffer	10 µL
DMSO	5 µL
dNTP mix (10 mM)	4 µL
RDS18F (50 µM)	2 µL
RSDDSMCSR (50 µM)	2 µL
Genomic DNA (50 ng/µL)	2 µL
Pfu enzyme (2.5 U/µL)	1 µL
DI water	74 µL
Total:	100 µL

Program	
94°C	2 minutes
8 cycles of:	
94°C	30 seconds
55°C	45 seconds
72°C	3 minutes
21 cycles of:	
94°C	30 seconds
61°C	45 seconds
72°C	3 minutes
72°C	7 minutes
4°C	Until used further

The PCR product was separated on a 1% TAE-agarose gel, and an about 1.8 Kb fragment was excised and gel purified. The isolated DNA was restricted with EcoRI and ClaI, and was column purified using a Qiagen gel isolation kit. Three µg of pMCS2rrnBP vector DNA was digested with EcoRI, and the linear DNA was gel isolated using a Qiagen gel isolation kit. The vector was further digested with ClaI, and the DNA was column purified. The double-digested vector was then dephosphorylated with shrimp alkaline phosphatase and purified using a QIAquick PCR Purification Kit. The EcoRI/ClaI-digested *R. sphaeroides* dds PCR product was ligated into the prepared vector using T4 DNA ligase for 14-16 hours at 16°C. One µL of the ligation reaction was transformed into *E. coli* Electromax™ DH10B™ cells, which were then plated on LBK (25 µg/mL) media. Individual colonies were resuspended in about 25 µL of DI water, and 2 µL of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 µL of the heated cells was used in a 25 µL PCR reaction using the RDS18F and RSDDSMCSR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 µM each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed under the following conditions: an initial denaturation at 94°C for 2 minutes; 6 cycles of 94°C for 30 seconds, 55°C for 45 seconds, and 72°C for 3 minutes; 25 cycles of 94°C for 30 seconds, 61°C 45 seconds, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. The resulting plasmid containing the Rsdds sequence under the control of the rrnB promotor was designated pMCS2rrnBP/Rsdds.

The pMCS2rrnBP/Rsdds plasmid was electroporated into *E. coli* strain S17-1. This strain contains a chromosomal copy of the trans-acting elements that mobilize oriT-containing plasmids during conjugation with a second bacterial strain. It also carries a gene conferring resistance to the antibiotics streptomycin and spectinomycin.

Using the S17-1 strain, the pMCS2rrnBP/Rsdds plasmid was transferred to *R. sphaeroides* 35053 by conjugation. Individual colonies were purified by restreaking on LBK plates. Single colonies were screened by PCR using the RDS18F and RSDDSMCSR primers to confirm the presence of the insert as described above.

pMCS2rrnBP/Stdds

The nucleic acid encoding a *S. trueperi* polypeptide having DDS activity was cloned in the pMCS2rrnBP vector as follows. The *S. trueperi* dds gene was PCR amplified using the following primer pair:

STDDSMCSF 5'-GTCGCTCGAGATCAGATAATCGTCGCTCAA-3' (SEQ ID NO:149)
STDDSMCSR 5'-ATATGGTACCGACATGGACGAGGAAGACGC-3' (SEQ ID NO:150)

The forward primer was located upstream of the start codon and introduced a XhoI restriction site, while the reverse primer was located downstream of the stop codon and introduced a KpnI restriction site. Since the forward primer was located upstream, the *S. trueperi* dds fragment maintained its native ribosomal binding site. The following reaction mix and PCR program were used to amplify the *S. trueperi* dds gene.

Reaction Mix	
Pfu 10X buffer	10 µL
DMSO	5 µL
dNTP mix (10 mM)	4 µL
SHDDSMCSF (50 µM)	2 µL
SHDDSMCSR (50 µM)	2 µL
Genomic DNA (50 ng/µL)	2 µL
Pfu enzyme (2.5 U/µL)	1 µL
DI water	74 µL

Program	
94°C	2 minutes
8 cycles of:	
94°C	30 seconds
55°C	45 seconds
72°C	3 minutes
21 cycles of:	
94°C	30 seconds
61°C	45 seconds

		72°C	3 minutes
Total:	100 µL	72°C	7 minutes
		4°C	Until used further

The PCR product was separated on a 1% TAE-agarose gel, and an about 1.6 Kb fragment was excised. The DNA was isolated using a Qiagen gel isolation kit. The isolated DNA was restricted with XhoI and KpnI, and was column purified using a Qiagen gel isolation kit. Two µg of pMCS2rrnBP vector DNA was digested with KpnI, and the linear DNA was gel isolated using a Qiagen gel isolation kit. The vector was further digested with XhoI, and the DNA was column purified. The double-digested vector was then dephosphorylated with shrimp alkaline phosphatase and column purified using a Qiagen gel purification kit. The XhoI/KpnI-digested *S. trueperi* dds PCR product was ligated into the prepared vector using T4 DNA ligase for 14-16 hours at 16°C. One µL of the ligation reaction was transformed into *E. coli* Electromax™ DH10B™ cells, which were then plated on LBK (25 µg/mL) media. Individual colonies were resuspended in about 25 µL of DI water, and 2 µL of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 µL of the heated cells was used in a 25 µL PCR reaction using the SHDDSMCSF and SHDDSMCSR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 µM each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed under the following conditions: an initial denaturation at 94°C for 2 minutes; 6 cycles of 94°C for 30 seconds, 55°C for 45 seconds, and 72°C for 3 minutes; 25 cycles of 94°C for 30 seconds, 61°C 45 seconds, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. The resulting plasmid containing the Stdds sequence under the control of the rrnB promotor was designated pMCS2rrnBP/Stdds.

The pMCS2rrnBP/Stdds plasmid was electroporated into *E. coli* strain S17-1. Using the S17-1 strain, the pMCS2rrnBP/Stdds plasmid was transferred to *R. sphaeroides* 35053 by conjugation. Individual colonies were purified by restreaking on LBK plates. Single colonies were screened by PCR using the SHDDSMCSF and SHDDSMCSR primers to confirm the presence of the insert as described above.

pMCS2glnBP/Rsdds

The nucleic acid encoding a *R. sphaeroides* polypeptide having DDS activity was cloned in the pMCS2glnBP vector as follows. The *R. sphaeroides* dds gene was PCR amplified using the following primer pair.

RSDDSF 5'-TAGAGAATTCGAAGGAAGAGCATGGGATTGGACG-AGGTTTC-3' (SEQ ID NO:151)

RSDDSR 5'-TACTACTTGTATGTAGGTACCACTTGCGGTCGGAC-TGATAG-3' (SEQ ID NO:152)

The forward primer introduced an EcoRI restriction site and a ribosomal binding site that was designed based on *R. sphaeroides* dxs1 gene. The reverse primer introduced a KpnI restriction site. Following reaction mix and PCR program was used to amplify the *R. sphaeroides* dds gene.

Reaction Mix	
Pfu 10X buffer	10 µL
DMSO	5 µL
dNTP mix (10 mM)	3 µL
RSDDSF (100 µM)	1 µL
RSDDSR (100 µM)	1 µL
Genomic DNA (50 ng/µL)	2 µL
Pfu enzyme (2.5 U/µL)	2 µL
DI water	76 µL
Total:	100 µL

Program	
94°C	2 minutes
7 cycles of:	
94°C	30 seconds
55°C	45 seconds
72°C	3 minutes
25 cycles of:	
94°C	30 seconds
62°C	45 seconds
72°C	3 minutes
72°C	7 minutes
4°C	Until used further

The PCR product was separated on a 1% TAE-agarose gel, and a fragment about 1.6 Kb in size was excised. The excised DNA was isolated using a Qiagen gel isolation kit. The isolated DNA was restricted with EcoRI and KpnI and was column purified using a Qiagen gel isolation kit. Three µg of pMCS2glnBP vector DNA was digested with KpnI, and the linear DNA was gel isolated using a Qiagen gel isolation kit. The vector was further digested with EcoRI, and the DNA was column purified. The double-digested vector was then dephosphorylated with shrimp alkaline phosphatase and column

purified using a Qiagen gel purification kit. The KpnI/EcoRI-digested *R. sphaeroides* dds PCR product with the *R. sphaeroides* dxs1 ribosomal binding site described above was ligated into the prepared vector using T4 DNA ligase for 14-16 hours at 16°C. One μL of the ligation reaction was transformed into *E. coli* Electromax™ DH10B™ cells, which were then plated on LBK (25 μg/mL) media. Individual colonies were resuspended in about 25 μL of DI water, and 2 μL of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μL of the heated cells was used in a 25 μL PCR reaction using the glnBF and RSDDSR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 μM each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed under the following conditions: an initial denaturation at 94°C for 2 minutes; 6 cycles of 94°C for 30 seconds, 55°C for 45 seconds, and 72°C for 3 minutes; 25 cycles of 94°C for 30 seconds, 62°C 45 seconds, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. A large scale plasmid preparation was done on a culture of a colony containing the Rsdds PCR product, and the glnBP/Rsdds region was sequenced to confirm the lack of nucleotide errors. The resulting plasmid containing the Rsdds sequence under the control of the glnB promotor was designated pMCS2glnBP/Rsdds.

The pMCS2glnBP/Rsdds plasmid DNA was electroporated into electrocompetent *R. sphaeroides* strain 35053 cells as well as electrocompetent carotenoid-deficient mutant cells of 35053 (ATCC 35053/ΔcrtE). Individual colonies of both strains were screened by PCR using the glnBF and RSDDSR primers to confirm the presence of the insert as described above.

pMCS2glnBP/Stdds

The nucleic acid encoding a *S. trueperi* polypeptide having DDS activity was cloned in the pMCS2glnBP vector as follows. The *S. trueperi* dds gene was PCR amplified using the following primer pair.

SHDDSECOVF 5'-GCGTGATATCGAAGGAAGAGCATGAGCGC-AACCGTCCACCG-3' (SEQ ID NO:153)

SHDDSKPNR 5'-ACTGCTAGGGTCCGAGGTACCGACATGGACGA-GGAAGACGC-3' (SEQ ID NO:154)

The forward primer introduced an EcoRV restriction site and a ribosomal binding site that was designed based on the *R. sphaeroides* dxs1 gene. The reverse primer introduced a KpnI restriction site. The following reaction mix and PCR program were used to amplify the *S. trueperi* dds gene.

Reaction Mix	
Pfu 10X buffer	10 µL
DMSO	5 µL
dNTP mix (10 mM)	3 µL
SHDDSECOVF (100 µM)	1 µL
SHDDSKPNR (100 µM)	1 µL
Genomic DNA (50 ng/µL)	2 µL
Pfu enzyme (2.5 U/µL)	2 µL
DI water	76 µL
Total:	100 µL

Program	
94°C	2 minutes
7 cycles of:	
94°C	30 seconds
58°C	45 seconds
72°C	3 minutes
25 cycles of:	
94°C	30 seconds
65°C	45 seconds
72°C	3 minutes
72°C	7 minutes
4°C	Until used further

The PCR product was separated on a 1% TAE-agarose gel, and a fragment about 1.2 Kb in size was excised. The excised DNA was isolated using a Qiagen gel isolation kit. The isolated DNA was restricted with EcoRV and KpnI and was column purified using a Qiagen gel isolation kit. Three µg of pMCS2glnBP vector DNA was digested with KpnI, and the linear DNA was gel isolated using a Qiagen gel isolation kit. The vector was further digested with EcoRV, and the DNA was column purified. The double-digested vector was then dephosphorylated with shrimp alkaline phosphatase and column purified using a Qiagen gel purification kit. The KpnI/EcoRV-digested *S. trueperi* dds PCR product with the *R. sphaeroides* dxs1 ribosomal binding site was ligated into the prepared vector using T4 DNA ligase for 14-16 hours at 16°C. One µL of the ligation reaction was transformed into *E. coli* Electromax™ DH10B™ cells, which were plated on LBK (25 µg/mL) media. Individual colonies were resuspended in about 25 µL of DI water, and 2 µL of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 µL of the heated

cells was used in a 25 μL PCR reaction using the glnBF and RSDDSR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 μM each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed under the following conditions: an initial denaturation at 94°C for 2 minutes; 6 cycles of 94°C for 30 seconds, 58°C for 45 seconds, and 72°C for 3 minutes; 25 cycles of 94°C for 30 seconds, 65°C 45 seconds, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. A large scale plasmid preparation was done on a culture of a colony containing the Stdds PCR product, and the glnBP/Stdds region was sequenced to confirm the lack of nucleotide errors. The resulting plasmid containing the Stdds sequence under the control of the glnB promotor was designated pMCS2glnBP/Stdds.

The pMCS2glnBP/Stdds plasmid DNA was electroporated into electrocompetent cells of *R. sphaeroides* strain 35053 and a carotenoid-deficient mutant of 35053 (ATCC 35053/ΔcrtE). Individual colonies of both strains were screened by PCR using the glnBF and SHDDSKPNR primers to confirm the presence of the insert as described above.

pMCS2tetP/Stdxs

The nucleic acid encoding a *S. trueperi* polypeptide having DXS activity was cloned in the pMCS2tetP vector as follows. The pMCS2tetP plasmid DNA was digested with the restriction enzyme KpnI, cleaned with a QIAquick PCR Purification Kit, and digested with the restriction enzyme ClaI. The enzyme reactions were inactivated by heating at 65°C for 20 minutes. The digested vector DNA was then dephosphorylated with shrimp alkaline phosphatase and gel purified on a 1% TAE-agarose gel. The Kpn1/ClaI-digested *S. trueperi* dxs PCR product described above with the *R. sphaeroides* dxs1 ribosomal binding site was ligated into the prepared vector using T4 DNA ligase for 16 hours at 16°C. One μL of the ligation reaction was transformed into *E. coli* Electromax™ DH5α™ cells, which were plated on LBK media. Individual colonies were resuspended in about 25 μL of 10 mM Tris, and 2 μL of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μL of the heated cells was used in a 25 μL PCR reaction using the SXSCLAF2 and SHDXSMCSR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 μM each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of

Taq DNA polymerase per reaction. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 54°C for 1 minute, and 72°C for 3.5 minutes; 24 cycles of 94°C for 30 seconds, 60°C 1 minute, and 72°C for 3.5 minutes; and a final extension for 7 minutes at 72°C. A large scale plasmid preparation was done on a culture of a colony containing the *S. trueperi* dxs PCR product, and the tetP/Stdxs region was sequenced to confirm the lack of nucleotide errors. The resulting plasmid containing the Stdxs sequence under the control of the tet promotor was designated pMCS2tetP/Stdxs.

Plasmid DNA (pMCS2tetP/Stdxs) was electroporated into electrocompetent cells of *R. sphaeroides* strain 35053 and a carotenoid-deficient mutant of 35053 (ATCC 35053/ΔcrtE). Individual colonies of both strains, along with an *E. coli* control, were screened by PCR using the TETXBAF and STDXSMCSR primers to confirm the presence of the insert as described above.

pMCS2tetP/Rsdds

The nucleic acid encoding a *R. sphaeroides* polypeptide having DDS activity was cloned in the pMCS2tetP vector as follows. Three μg of plasmid DNA of the pMCS2tetP vector was digested with the restriction enzyme KpnI. The digested DNA was cleaned with a QIAquick PCR Purification Kit and digested with the restriction enzyme EcoRI, after which the enzyme was inactivated by heating at 65°C for 20 minutes. The digested vector DNA was then dephosphorylated with shrimp alkaline phosphatase and gel purified. Sixty ng of vector DNA was ligated with 120 ng of the KpnI/EcoR I-digested *R. sphaeroides* dds PCR product described above using T4 DNA ligase at 16°C for 16 hours. One μL of the ligation reaction was transformed into *E. coli* Electromax™ DH5α™, which were then plated on LBK media. Individual colonies were resuspended in about 25 μL of 10 mM Tris, and 2 μL of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μL of the heated cells used in a 25 μL PCR reaction using the TETXBAF and RSDDSMCSR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 μM each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed in a MJ Research PTC100

under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 55°C for 1 minute, and 72°C for 3 minutes; 24 cycles of 94°C for 30 seconds, 64°C 1 minute, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. Plasmid DNA was isolated for a colony having the desired insert, and the tetP/Rsdds region was sequenced to confirm the lack of nucleotide errors from PCR. The resulting plasmid containing the Rsdds sequence under the control of the tet promotor was designated pMCS2tetP/Rsdds.

Plasmid DNA (pMCS2tetP/Rsdds) was electroporated into electrocompetent cells of *R. sphaeroides* strain 35053 and the ATCC 35053/ΔcrtE strain. Individual colonies of both strains, along with an *E. coli* control, were screened by PCR using the TETXBAF and RSDDSMCSR primers to confirm the presence of the insert as described above.

pMCS2tetP/Stdds

The nucleic acid encoding a *S. trueperi* polypeptide having DDS activity was cloned in the pMCS2tetP vector as follows. Three μg of pMCS2tetP plasmid DNA was digested with the restriction enzyme KpnI. The digested DNA was gel purified and digested with the restriction enzyme EcoRV. The enzyme was then inactivated by heating at 80°C for 20 minutes, and the DNA dephosphorylated with shrimp alkaline phosphatase. The dephosphorylated DNA was purified using a QIAquick PCR purification kit. Fifty μg of digested vector DNA was ligated with 150 ng of the KpnI/EcoRV-digested *S. trueperi* dds PCR product described above using T4 DNA ligase at 16°C for 16 hours. One μL of the ligation reaction was transformed into *E. coli* Electromax™ DH10B™ cells, which were then plated on LBK media. Individual colonies were resuspended in about 25 μL of 10 mM Tris, and 2 μL of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μL of the heated cells used in a 25 μL PCR reaction using the TETXBAF and STDDSMCSR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 μM each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2

minutes; 8 cycles of 94°C for 30 seconds, 55°C for 1 minute, and 72°C for 3 minutes; 24 cycles of 94°C for 30 seconds, 64°C for 1 minute, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. Plasmid DNA was isolated for a colony having the desired insert and was sequenced in the tetP/Stdds region to confirm the DNA sequence of the insert. The resulting plasmid containing the Stdds sequence under the control of the tet promotor was designated pMCS2tetP/Stdds.

Plasmid DNA (pMCS2tetP/Stdds) was electroporated into electrocompetent cells of *R. sphaeroides* strain 35053 and the ATCC 35053/ΔcrtE strain. Individual colonies of both strains, along with an *E. coli* control, were screened by PCR using the TETXBAF and STDDSMCSR primers to confirm the presence of the insert as described above.

pMCS2tetP/Stdxs/Rsdds

Nucleic acid encoding a *S. trueperi* polypeptide having DXS activity as well as nucleic acid encoding a *R. sphaeroides* polypeptide having DDS activity was cloned into the pMCS2tetP vector as follows. A vector containing both the *S. trueperi* dxs gene and the *R. sphaeroides* dds gene, each behind a tet promoter, was constructed using the pMCS2tetP/Stdxs construct described above as the starting vector. This vector was digested with restriction enzyme XbaI, cleaned with a QIAquick PCR Purification Kit, and digested with the restriction enzyme Bpu10I (Fermentas, Hanover, MD). The enzyme reaction was inactivated by heating for 20 minutes at 80°C. The digested vector DNA was then dephosphorylated using shrimp alkaline phosphatase and gel purified on a 1% TAE-agarose gel.

A PCR product containing a tet promoter region followed by a *R. sphaeroides* dds gene was amplified using the pMCS2tetP/Rsdds construct described above as template. The PCR mix contained the following: 1X Native Plus Pfu buffer, 5 ng plasmid template, 0.2 μM each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 10 units of native Pfu DNA polymerase in a final volume of 200 μL. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 55°C for 1 minute, and 72°C for 3 minutes; 24 cycles of 94°C for 30 seconds, 64°C 1 minute, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. The amplification product was then separated by gel

electrophoresis using a 1% TAE-agarose gel. A 1.6 Kb fragment was excised from the gel and purified. The purified fragment was digested with Bpu10I, cleaned with a QIAquick PCR Purification Kit, digested with Xba I restriction enzyme, purified again with a QIAquick PCR Purification Kit, and quantified on a minigel.

60 ng of the prepared pMCS2tetP/Stdxs vector was ligated with 70 ng of the digested tetP/Rsdds PCR product using T4 DNA ligase at 16°C for 16 hours. One μL of ligation reaction was used to electroporate 40 μL of *E. coli* Electromax™ DH5α™ cells. Electroporated cells were plated on LBK media. Individual colonies were screened by PCR using the RSDDSMCSF and STDXSMCSR primers, which produced a 4.1 Kb band. Individual colonies were resuspended in about 25 μL of 10 mM Tris, and 2 μL of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μL of the heated cells used in a 25 μL PCR reaction. The PCR reaction mix contained 0.2 μM each primer, 1X Genome Advantage (Clontech, Palo Alto, CA) reaction buffer, 1 M GCMelt, 1.1 mM $Mg(OAc)_2$, 0.2 mM each dNTP, and 1X Genome Advantage Polymerase. The PCR was conducted in a MJ Research PTC100 and consisted of an initial denaturation at 94°C for 1.5 minutes; 32 cycles of a 30 second denaturation at 94°C, a 1 minute annealing at 60°C, and a 6.5 minute extension at 72°C; followed by a final extension at 72°C for 5 minutes. A large-scale plasmid prep was done for a colony that had the desired insert, and plasmid DNA was sequenced through the tetP/Rsdds region to confirm the lack of nucleotide errors from PCR. The resulting plasmid containing the Stdxs sequence under the control of the tet promotor and the Rsdds sequence under the control of the tet promotor was designated pMCS2tetP/Stdxs/Rsdds.

Plasmid DNA (pMCS2tetP/Stdxs/Rsdds) was electroporated into electrocompetent cells of *R. sphaeroides* strains 35053 and the ATCC 35053/ΔcrtE. Individual colonies of both strains, along with an *E. coli* control, were screened by PCR using the RSDDSMCSF and STDDSMCSR primers to confirm the presence of the insert as described above.

pMCS2tetP/Stdxr

Nucleic acid encoding a *S. trueperi* polypeptide having DXR activity was cloned into the pMCS2tetP vector as follows. The *S. trueperi* dxr gene was amplified using genomic DNA as template. The following primers were designed to introduce an EcoRV restriction site and a ribosomal binding based on *R. sphaeroides* dxs1 gene at the beginning of the amplified fragment and a KpnI site at the end of the amplified fragment.

SXRRVF 5'-GATGATATCGAAGGAAGAGCATGGTGAAGCGCGT-CACGGTGT-3' (SEQ ID NO:155)

SXRKPNR 5'-CAAGAGTCAGAAGGTACCCGCCAGAATGGTGAGC-AGGATG-3' (SEQ ID NO:156)

The PCR mix contained the following: 1X Native Plus Pfu buffer, 200 ng genomic DNA, 0.2 μM of each primer, 0.2 mM of each dNTP, 5% DMSO (v/v), and 10 units of native Pfu DNA polymerase in a final volume of 200 μL. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 59°C for 1 minute, and 72°C for 3 minutes; 24 cycles of 94°C for 30 seconds, 64°C 1 minute, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. The amplification product was then separated by gel electrophoresis using a 1 % TAE-agarose gel. A 1.0 Kb fragment was excised from the gel and purified. The purified fragment was digested simultaneously with EcoRV and KpnI restriction enzymes, purified with a QIAquick PCR Purification Kit, and checked on a minigel.

Fifty ng of the EcoRV, KpnI-digested pMCS2tetP vector described above for the pMCS2tetP/Stdds construct was ligated with 75 ng of the digested *S. trueperi* dxr PCR product using T4 DNA ligase at 20°C for 4 hours. One μL of ligation reaction was used to electroporate 40 μL of *E. coli* Electromax™ DH10B™ cells, which were then plated on LBK media. Individual colonies were selected and screened by PCR using the TETXBAF and SXRKPNR primers. The PCR mix contained the following: 1X Taq PCR buffer, 200 ng genomic DNA, 0.2 μM of each primer, 0.2 mM of each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per 25 μL reaction. The PCR reaction was

performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 32 cycles of 94°C for 30 seconds, 64°C 1 minute, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. A large-scale plasmid preparation was done for a colony that had the desired insert, and the tetP/Stdxr region was sequenced to confirm the DNA sequence of the insert. The resulting plasmid containing the Stdxr sequence under the control of the tet promotor was designated pMCS2tetP/Stdxr.

Plasmid DNA (pMCS2tetP/Stdxr) was electroporated into electrocompetent cells of *R. sphaeroides* strains 35053 and ATCC 35053/ΔcrtE. Individual colonies of both strains, along with an *E. coli* control, were screened by PCR using the TETXBAF and SXRKPNR primers to confirm the presence of the insert as described above.

pMCS2tetP/Stdxr/Stdds

Nucleic acid encoding a *S. trueperi* polypeptide having DXR activity as well as nucleic acid encoding a *S. trueperi* polypeptide having DDS activity was cloned into the pMCS2tetP vector as follows. A vector containing both the *S. trueperi* dxr and dds genes, each behind a tet promoter, was constructed using the pMCS2tetP/Stdds construct described above as the starting vector. This vector was digested with restriction enzyme XbaI, cleaned with a QIAquick PCR Purification Kit, and digested with the restriction enzyme Bpu10I (Fermentas). The enzyme reaction was inactivated by heating for 20 minutes at 80°C. The digested vector DNA was then dephosphorylated with shrimp alkaline phosphatase and gel purified.

A PCR product containing a tet promoter region followed by a *S. trueperi* dxr gene was amplified using the pMCS2tetP/Stdxr construct described above as template and primers TETBPUF and SXRXBAR. The SXRXBAR primer, having the following sequence, was designed to introduce an XbaI restriction site on the end of the PCR product.

SXRXBAR 5'-CAAGAGTCAGAATCTAGACGCCAGAATGGTGA-
GCAGGATG-3' (SEQ ID NO:157)

The PCR mix contained the following: 1X Native Plus Pfu buffer, 5 ng plasmid template, 0.2 μM each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 10 units of native Pfu DNA polymerase in a final volume of 200 μL. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 59°C for 1 minute, and 72°C for 3.5 minutes; 24 cycles of 94°C for 30 seconds, 64°C 1 minute, and 72°C for 3.5 minutes; and a final extension for 7 minutes at 72°C. The amplification product was then separated by gel electrophoresis using a 1% TAE-agarose gel. A 1.4 Kb fragment was excised from the gel and purified. The purified fragment was digested with Bpu10I, cleaned with a QIAquick PCR Purification Kit, digested with XbaI restriction enzyme, purified again with a QIAquick PCR Purification Kit, and quantified on a minigel.

Sixty ng of the prepared pMCS2tetP/Stdds vector was ligated with 80 ng of the digested tetP/Stdxr PCR product using T4 DNA ligase at 16°C for 16 hours. One μL of ligation reaction was used to electroporate 40 μL of *E. coli* Electromax™ DH10B™ cells, which were then plated on LBK media. Individual colonies were screened by PCR using the SXREVF and SDSKPNR primers. Colonies were resuspended in about 25 μL of 10 mM Tris, and 2 μL of the resuspension was plated on LBK media. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μL of the heated cells used in a 25 μL PCR reaction. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 μM each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 58°C for 1 minute, and 72°C for 4.5 minutes; 24 cycles of 94°C for 30 seconds, 64°C 1 minute, and 72°C for 4.5 minutes; and a final extension for 7 minutes at 72°C. A large-scale plasmid preparation was done for a colony that had the desired insert, and the tetP/Stdxr region was sequenced to confirm the lack of nucleotide errors from PCR. The resulting plasmid containing the Stdxr sequence under the control of the tet promotor and the Stdds sequence under the control of the tet promotor was designated pMCS2tetP/Stdxr/Stdds.

Plasmid DNA (pMCS2tetP/Stdxr/Stdds) was electroporated into electrocompetent cells of *R. sphaeroides* strains 35053 and ATCC 35053/ΔcrtE. Individual colonies of

both strains, along with an *E. coli* control, were screened by PCR using the SXREVF and SDSKPNR primers to confirm the presence of the insert as described above.

pMCS2tetP/EcUbiC

Nucleic acid encoding a *E. coli* polypeptide having chorismate lyase activity was cloned into the pMCS2tetP vector as follows. The *E. coli* ubiC gene was amplified using genomic DNA from *E. coli* strain DH10B as template. The following primers were designed to introduce an EcoRV restriction site and a ribosomal binding site based on *R. sphaeroides* dxs1 gene at the beginning of the amplified fragment, and a KpnI site at the end of the amplified fragment.

UBICRVF 5'-CTAGATATCGGAAGGAAGAGCATGTCACAC-CCCGCGTTA-3' (SEQ ID NO:158)

UBICKPNR 5'-TCAGGTACCGTGTCGCCACCCACAACGCC-CATAATG-3' (SEQ ID NO:159)

The PCR mix contained the following: 1X Native Plus Pfu buffer, 200 ng genomic DNA, 0.2 μM each primer, 0.2 mM each dNTP, and 10 units of native Pfu DNA polymerase in a final volume of 200 μL. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 57°C for 1 minute, and 72°C for 2.5 minutes; 24 cycles of 94°C for 30 seconds, 64°C 1 minute, and 72°C for 2.5 minutes; and a final extension for 7 minutes at 72°C. The amplification product was then separated by gel electrophoresis using a 1.5 % TAE-agarose gel. A 650 bp fragment was excised from the gel and purified. The purified fragment was digested with EcoRV, cleaned with a QIAquick PCR Purification Kit, digested with KpnI restriction enzyme, purified again with a QIAquick PCR Purification Kit, and quantified on a minigel.

Seventy-five ng of the EcoRV, KpnI-digested pMCS2tetP vector described above for the pMCS2tetP/Stdds construct was ligated with 70 ng of the digested ubiC PCR product using T4 DNA ligase at 16°C for 16 hours. One μL of ligation reaction was used to electroporate 40 μL of *E. coli* Electromax™ DH5α™ cells, which were then plated on

LBK media. Individual colonies were resuspended in about 25 μL of 10 mM Tris, and 2 μL of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μL of the heated cells used in a 25 μL PCR reaction using the TETXBAF and UBICKPNR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 μM each primer, 0.2 mM each dNTP, and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 32 cycles of 94°C for 30 seconds, 62°C for 1 minute, and 72°C for 2 minutes; and a final extension for 7 minutes at 72°C. A large-scale plasmid preparation was done for a colony that had the desired insert and the tetP/ubiC region was sequenced to confirm the DNA sequence of the insert. The resulting plasmid containing the UbiC sequence under the control of the tet promotor was designated pMCS2tetP/EcUbiC.

Plasmid DNA (pMCS2tetP/EcUbiC) was electroporated into electrocompetent cells of *R. sphaeroides* strain 35053 and the ATCC 35053/ΔcrtE strain. Individual colonies of both strains, along with an *E. coli* control, were screened by PCR using the TETXBAF and UBICKPNR primers to confirm the presence of the insert as described above with the addition of 5% DMSO (v/v) to the PCR reaction.

pMCS2tetP/Stdxs/Rsdds/EcUbiC

Nucleic acid encoding an *S. trueperi* polypeptide having DXS activity, nucleic acid encoding an *R. sphaeroides* polypeptide having DDS activity, and nucleic acid encoding an *E. coli* polypeptide having chorismate lyase activity was cloned into the pMCS2tetP vector as follows. A vector containing the *S. trueperi* dxs gene, the *R. sphaeroides* dds gene, and the *E. coli* ubiC gene, each behind a tet promoter, was constructed using the pMCS2tetP/Stdxs/Rsdds construct described above as the starting vector. This vector was digested with restriction enzyme KpnI, cleaned with a QIAquick PCR Purification Kit, and digested with the restriction enzyme NsiI. The enzyme reaction was inactivated by heating for 20 minutes at 65°C. The digested vector DNA was then dephosphorylated with shrimp alkaline phosphatase and gel purified.

A PCR product containing a tet promoter region followed by an *E. coli* ubiC gene was amplified using the pMCS2tetP/EcUbiC construct described above as template. The

following primers were designed to introduce an KpnI restriction site at the beginning of the amplified fragment and an NsiI site at the end of the amplified fragment.

TETKPNF 5'-TAGGGTACCACCGTCTACGCCGACCT-CGTTCAAC-3' (SEQ ID NO:160)

UBICNSIR 5'-TGTATGCATGTCGCCACCCACAACGC-CCATAATG-3' (SEQ ID NO:161)

The PCR mix contained the following: 1X Native Plus Pfu buffer, 5 ng plasmid template, 0.2 μM each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 10 units of native Pfu DNA polymerase in a final volume of 200 μL. The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 62°C for 1 minute, and 72°C for 2.5 minutes; 24 cycles of 94°C for 30 seconds, 66°C 1 minute, and 72°C for 2.5 minutes; and a final extension for 7 minutes at 72°C. The amplification product was then separated by gel electrophoresis using a 1% TAE-agarose gel. An 850 bp fragment was excised from the gel and purified. The purified fragment was digested with the restriction enzyme NsiI, cleaned with a QIAquick PCR Purification Kit, digested with the restriction enzyme KpnI, purified again with a QIAquick PCR Purification Kit, and quantified on a minigel.

Fifty ng of the prepared pMCS2tetP/Stdxs/Rsdds vector was ligated with 35 ng of the digested tetP/ubiC PCR product using T4 DNA ligase at 16°C for 16 hours. One μL of ligation reaction was used to electroporate 40 μL of *E. coli* Electromax™ DH10B™ cells, which were then plated on LBK media. Individual colonies were resuspended in about 25 μL of 10 mM Tris, and 2 μL of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μL of the heated cells used in a 25 μL PCR reaction using the SXSCLAF2 and UBICNSIR primers. The PCR reaction mix contained 1X GC-RICH PCR reaction buffer, 1.0 M GC-RICH resolution solution, 0.2 μM each primer, 0.2 mM each dNTP, and 1 unit of GC-RICH enzyme mix per reaction (Roche). The PCR reaction was performed in a MJ Research PTC100 under the following conditions: an initial

denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 60°C for 1 minute, and 72°C for 5 minutes; 24 cycles of 94°C for 30 seconds, 64°C 1 minute, and 72°C for 5 minutes; and a final extension for 7 minutes at 72°C. A large-scale plasmid preparation was done for a colony that had the desired insert, and plasmid DNA was sequenced through the tetP/ubiC region to confirm the lack of nucleotide errors from PCR. The resulting plasmid containing Stdxs sequence under the control of the tet promotor, the Rsdds sequence under the control of the tet promotor, and the UbiC sequence under the control of the tet promotor was designated pMCS2tetP/Stdxs/Rsdds/EcUbiC.

Plasmid DNA (pMCS2tetP/Stdxs/Rsdds/EcUbiC) was electroporated into electrocompetent cells of *R. sphaeroides* strains 35053 and ATCC 35053/ΔcrtE. Individual colonies of both strains, along with an *E. coli* control, were screened by PCR using the SXSCLAF2 and UBICNSIR primers to confirm the presence of the insert as described above.

pMCS2tetP/RsLytB

Nucleic acid encoding a LytB *R. sphaeroides* polypeptide was cloned into the pMCS2tetP vector as follows. The *R. sphaeroides* lytB was identified by TBLASTN analysis of its genome using an *E. coli* lytB sequence as a query. Based on the identified sequence the following primers were designed to PCR amplify the gene:

LYTBHINDF 5'-GACGAAGCTTGAAGGAAGAGCATGCCTCCCCTCA-CCCTCTATC-3' (SEQ ID NO:162)

LYTBKPNR 5'-GTCACTGAATGAATGGTACCGCAGCCGAGAACCG-CCAGAAGCC-3' (SEQ ID NO:163)

The primers introduced a HindIII restriction site and ribosomal binding site at the 5' end, and a KpnI restriction site at the 3' end. The following reaction mix and PCR program were used to amplify the lytB gene.

Reaction Mix		Program
Pfu 10X buffer	10 μL	94°C 2 minutes
DMSO	5 μL	7 cycles of:

dNTP mix (10 mM)	3 µL	94°C	30 seconds
LYTBHINDF (100 µM)	1 µL	59°C	45 seconds
LYTBKPNR (100 µM)	1 µL	72°C	3 minutes
Genomic DNA (50 ng/µL)	2 µL	25 cycles of:	
Pfu enzyme (2.5 U/µL)	2 µL	94°C	30 seconds
DI water	76 µL	66°C	45 seconds
		72°C	3 minutes
Total:	100 µL	72°C	7 minutes
		4°C	Until used further

The PCR product was run on a 1% TAE-agarose gel, and a fragment about 1.1 Kb in size was excised. The excised DNA was isolated using a Qiagen gel isolation kit. The isolated DNA was restricted with HindIII and KpnI, and was column purified using a Qiagen gel isolation kit. Two µg of pMCS2tetP vector DNA was digested with HindIII, and the linear DNA was gel isolated using a Qiagen gel isolation kit. The vector was further digested with KpnI, and the DNA was column purified. The double-digested vector was then dephosphorylated with shrimp alkaline phosphatase and column purified using a Qiagen gel purification kit. The KpnI/HindIII-digested *R. sphaeroides* lytB PCR product with the *R. sphaeroides* dxs1 ribosomal binding site described above was ligated into the prepared vector using T4 DNA ligase for 14-16 hours at 16°C. One µL of the ligation reaction was transformed into *E. coli* Electromax™ DH10B™ cells, which were then plated on LBK (25 µg/mL) media. Individual colonies were resuspended in about 25 µL of DI water, and 2 µL of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 µL of the heated cells was used in a 25 µL PCR reaction using the LYTBHINDF and LYTBKPNR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 µM each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed under the following conditions: an initial denaturation at 94°C for 2 minutes; 8 cycles of 94°C for 30 seconds, 59°C for 1 minute, and 72°C for 3 minutes; 24 cycles of 94°C for 30 seconds, 66°C for 1 minute, and 72°C for 3 minutes; and a final extension for 7 minutes at 72°C. A large scale plasmid preparation was done on a culture of a colony containing the lytB PCR product, and the tetP/lytB region was sequenced to confirm the lack of nucleotide errors.

The resulting plasmid containing the RsLytB sequence under the control of the tet promotor was designated pMCS2tetP/RsLytB.

Plasmid DNA (pMCS2tetP/RsLytB) was electroporated into electrocompetent cells of *R. sphaeroides* strain 35053 and a carotenoid-deficient mutant of 35053 (ATCC 35053/ΔcrtE). Individual colonies of both strains, along with an *E. coli* control, were screened by PCR using the TETXBAF and LYTBKPNR primers to confirm the presence of the insert as described above.

pMCS2tetP/Stdxs/Rsdds/RsLytB

Nucleic acid encoding an *S. trueperi* polypeptide having DXS activity, nucleic acid encoding an *R. sphaeroides* polypeptide having DDS activity, and nucleic acid encoding LytB from *R. sphaeroides* were cloned into the pMCS2tetP vector as follows. The *R. sphaeroides* lytB gene was cloned and expressed along with the *R. sphaeroides* dds and *S. trueperi* dxs genes. In this triple expression system, each gene was expressed through its own tetP. The *R. sphaeroides* lytB gene was PCR amplified along with the tetP using the following primers.

TETKPNF 5'-TAGGGTACCACCGTCTACGCCGACCTC-GTTGAAC-3' (SEQ ID NO:164)

LYTBNSIR 5'-AGGCAATGCATGCAGCCGAGAACCGCC-AGAAGCC-3' (SEQ ID NO:165)

The following PCR mix and program were used to PCR amplify the lytB gene along with the tetP.

Reaction Mix	
Pfu 10X buffer	10 µL
DMSO	5 µL
dNTP mix (10 mM)	3 µL
TETKPNF (100 µM)	1 µL
LYTBNSIR (100 µM)	1 µL
pMCS2tetP/lytB (10 ng/µL)	1 µL
Pfu enzyme (2.5 U/µL)	2 µL
DI water	77 µL

Program	
94°C	2 minutes
7 cycles of:	
94°C	30 seconds
63°C	45 seconds
72°C	3 minutes
25 cycles of:	
94°C	30 seconds
69°C	45 seconds

		72°C	3 minutes
Total:	100 µL	72°C	7 minutes
		4°C	Until used further

In this PCR reaction, pMCS2tetP/RsLytB plasmid DNA was used as a template. The PCR product was separated on a 1% TAE-agarose gel, and a fragment about 1.4 Kb in size was excised. The excised DNA was isolated using a Qiagen gel isolation kit. The isolated DNA was restricted with NsiI and KpnI, and was column purified using a Qiagen gel isolation kit. Two µg of pMCS2tetP/Stdxs/Rsdds plasmid DNA was digested with NsiI, and the linear DNA was gel isolated using a Qiagen gel isolation kit. The vector was further digested with KpnI, and the DNA was column purified. The double-digested vector was then dephosphorylated with shrimp alkaline phosphatase and column purified using a Qiagen gel purification kit. The KpnI/NsiI-digested PCR product was ligated into the prepared plasmid using T4 DNA ligase for 14-16 hours at 16°C. One µL of the ligation reaction was transformed into *E. coli* Electromax™ DH10B™ cells, which were then plated on LBK (25 µg/mL) media. Individual colonies were resuspended in about 25 µL of DI water, and 2 µL of the resuspension was plated on LBK. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 µL of the heated cells was used in a 25 µL PCR reaction using the SXSCLAF2 and LYTBNSIR primers. The PCR mix contained the following: 1X Taq PCR buffer, 0.2 µM each primer, 0.2 mM each dNTP, 5% DMSO (v/v), and 1 unit of Taq DNA polymerase per reaction. The PCR reaction was performed under the following conditions: an initial denaturation at 94°C for 2 minutes; 6 cycles of 94°C for 30 seconds, 59°C for 45 sec, and 72°C for 4 minutes; 25 cycles of 94°C for 30 seconds, 65°C for 45 seconds, and 72°C for 4 minutes; and a final extension for 7 minutes at 72°C. A large scale plasmid preparation was done on a culture of a colony containing the correct insert, and the tetP/lytB region was sequenced to confirm the lack of nucleotide errors. The resulting plasmid containing Stdxs sequence under the control of the tet promotor, the Rsdds sequence under the control of the tet promotor, and the LytB sequence under the control of the tet promotor was designated pMCS2tetP/Stdxs/Rsdds/RsLytB.

Plasmid DNA (pMCS2tetP/Stdxs/Rsdds/RsLytB) was electroporated into electrocompetent cells of *R. sphaeroides* strain 35053 and a carotenoid-deficient mutant

of 35053 (ATCC 35053/ΔcrtE). Individual colonies of both strains were screened by PCR using the SXSCLAF2 and LYTBNSIR primers to confirm the presence of the insert as described above.

Example 9 – Making recombinant microorganisms containing knock-outs

Various nucleic acid sequences within the *R. sphaeroides* genome were knocked out. All restriction enzymes and T4 DNA ligase were obtained from New England Biolabs (Beverly, MA) unless otherwise indicated. All plasmid DNA preparations were done using QIAprep Spin Miniprep Kits or Qiagen Maxi Prep Kits, and all gel purifications were done using QIAquick Gel Extraction Kits (Qiagen, Valencia, CA).

ATCC 35053/ΔcrtE(kan)

R. sphaeroides cells lacking crtE were made by inserting a kanamycin resistance gene into the crtE sequence as follows. In general, the crtE gene from *R. sphaeroides* was cloned into a pUC19 vector, and a kanamycin gene (kan) was inserted into the gene to inactivate it. The crtE-kan insert was amplified by PCR and cloned into pSUP203, a mobilizable ColE1-based plasmid that is not maintained in *R. sphaeroides* unless it is integrated into a *R. sphaeroides* replicon. This plasmid was transformed into *E. coli* strain S17-1, a strain that is able to mobilize oriT-containing plasmids in conjugations with a second bacterial strain. The S17-1 strain was conjugated with *R. sphaeroides* strain 35053, and colonies were identified in which the crtE-kan insert had replaced the native crtE gene.

The crtE gene from *R. sphaeroides* strain 17023 was amplified by PCR using primers designed to introduce an SphI restriction site at the beginning of the amplified fragment and an XbaI restriction site at the end of the amplified fragment. The sequences of the primers were as follows.

CRTESPHF 5'-AAGCATGCGAAAAAGTTGACACCTGTGGAGTC-3' (SEQ ID NO:166)

CRTEXBAR 5'-ACTCTAGAAGCACCTGCGAATGGACGAAG-3' (SEQ ID NO:167)

The fragment amplified included the crtE gene along with 85 nucleotides upstream of the translational start codon and 228 nucleotides downstream of the translational stop codon. The PCR reaction mix contained 0.2 μM each primer, 1X GC Genomic PCR Buffer (Clontech, Palo Alto, CA), 1 M GC-Melt, 1.1 mM $Mg(OAc)_2$, 0.2 mM each dNTP, 1X Advantage-GC Genomic Polymerase Mix, and 1 ng of genomic DNA per μL of reaction mix. The PCR was conducted in a Perkin Elmer Geneamp 2400 and consisted of an initial denaturation at 94°C for 30 seconds; 35 cycles of a 15 second denaturation at 94°C, a one minute annealing at 55°C, and a 3 minute extension at 72°C; followed by a final extension at 72°C for 5 minutes. Fifty μL of PCR product was separated on a 1% Tris-Acetate-EDTA (TAE)-agarose gel. A 1180 bp fragment was gel purified, and the purified DNA was digested with XbaI and SphI restriction enzymes (Promega, Madison, WI).

pUC19 vector was digested with the restriction enzymes SphI and XbaI, and gel purified on a 1% TAE- agarose gel. Fifty ng of purified vector was ligated with about 150 ng of digested crtE PCR product for 16 hours at 14°C using T4 DNA ligase (Roche Molecular Biochemicals, Indianapolis, IN). One μL of ligation reaction was transformed into ElectroMAX™ DH10B™ cells (Life Technologies, Gaithersburg, MD), which were then plated on LB media containing 100 μg/mL ampicillin and 50 μg/mL of 5-Bromo-4-Chloro-3-Indolyl-B-D-Galactopyranoside (LBKX). Individual, white colonies were resuspended in about 20 μL of 10 mM Tris, and 2 μL of the resuspension was plated on LBKX media. The remnant resuspension was heated for 10 minutes at 95°C to break open the bacterial cells, and 2 μL of the heated cells was used in a 25 μL PCR reaction using the CRTESPHF and CRTEXBAR primers. The PCR reaction mix contained 0.2 μM each primer, 1X GC Genomic PCR Buffer, 1 M GCMelt, 1.1 mM $Mg(OAc)_2$, 0.2 mM each dNTP, and 1X Advantage-GC Genomic Polymerase Mix. The PCR was conducted in a Perkin Elmer Geneamp 2400 and consisted of an initial denaturation at 94°C for 30 seconds; 35 cycles of a 15 second denaturation at 94°C, a one minute annealing at 55°C, and a 3 minute extension at 72°C; followed by a final extension at 72°C for 5 minutes. Plasmid DNA was isolated for colonies having a crtE gene insert and was digested with the restriction enzyme HindIII and with a mixture of SphI and XbaI to confirm vector structure.

One μg of the pUC19crtE construct was digested with XhoI and StuI restriction enzymes. These enzymes cut a 273 bp fragment of DNA from the center of the crtE gene. The digested DNA was separated on a 1% TAE-agarose gel. A 3.6 Kb fragment representing pUC19 and the remaining ends of the crtE gene was excised and purified.

The kanamycin resistance gene was amplified by PCR from the PCRII vector (Invitrogen, Carlsbad, CA) using primers designed to introduce an StuI restriction site at the beginning of the amplified fragment and an XhoI restriction site at the end of the amplified fragment. The sequences of the primers were as follows.

KANSTUF 5'-ATAAAGGCCTTACATGGCGATAGCTAGACTG-3' (SEQ ID NO:168)
KANXHOR 5'-AAGGCTCGAGAAGGATCTTACCGCTGTTGAG-3' (SEQ ID NO:169)

The PCR reaction mix contained 0.2 μM each primer, 1X Pfu reaction buffer (Stratagene, La Jolla, CA), 0.2 mM each dNTP, 8 units Pfu, and 5 ng of the PCRII vector in a 200 μL reaction. The PCR was conducted in a Perkin Elmer Geneamp 2400 and consisted of an initial denaturation at 94°C for 2 minutes; 8 cycles of a 30 second denaturation at 94°C, a 1 minute annealing at 55°C, and a 2.5 minute extension at 72°C; 24 cycles of a 30 second denaturation at 94°C, a 1 minute annealing at 55°C, and a 2.5 minute extension at 72°C; followed by a final extension at 72°C for 5 minutes. The PCR product was separated on a 1% TAE- agarose gel, and a 1.2 Kb fragment was excised and purified. One μg of purified DNA was digested with XhoI and StuI restriction enzymes and cleaned using a QIAquick PCR Purification Kit.

Fifty ng of the digested pUC19crtE vector DNA was ligated with 75 ng of the digested kan PCR product for 16 hours at 14°C using T4 DNA ligase (Roche). One μL of ligation mix was electroporated into 40 μL of *E. coli* ElectroMAX™ DH10B™ electrocompetent cells, which were then plated on LB media containing 100 μg/mL ampicillin and 50 μg/mL kanamycin (LBAK). Plasmid DNA was isolated from cultures of individual colonies and was digested in separate reactions with the restriction enzymes PstI, SphI, and a StuI/XbaI mixture to confirm correct vector structure.

The crtE gene with the inserted kan gene was amplified by PCR using primers designed to have ScaI restriction sites on both ends of the fragment. The sequences of the primers were as follows.

CRTESCAF 5'-ATAGTACTGAAAAAGTTGACACCTGTGGAGTC-3' (SEQ ID NO:170)
CRTESCAR 5'-ATAGTACTAGCACCTGCGAATGGACGAAG-3' (SEQ ID NO:171)

The PCR reaction mix contained 0.2 μM each primer, 1X GC Genomic PCR Buffer, 1 M GCMelt, 1.1 mM $Mg(OAc)_2$, 0.2 mM each dNTP, 1X Advantage-GC Genomic Polymerase Mix, and 1 ng of plasmid DNA per μL of reaction mix. The PCR was conducted in a Perkin Elmer Geneamp 9600 and consisted of an initial denaturation at 94°C for 1 minute; 8 cycles of a 30 second denaturation at 94°C, a 1 minute annealing at 55°C, and a 4 minute extension at 72°C; 25 cycles of a 30 second denaturation at 94°C, a 1 minute annealing at 60°C, and a 4 minute extension at 72°C; followed by a final extension at 72°C for 5 minutes. 200 μL of PCR product was separated on a 1% TAE-agarose gel. A 2.0 Kb fragment was excised and purified. One μg of purified DNA was digested with ScaI restriction enzyme, and the digested DNA was purified using a QIAquick PCR Purification Kit.

2.3 μg of pSUP203 plasmid DNA was digested with ScaI restriction enzyme. The digested DNA was separated on a 1% TAE-agarose gel, and a 7.6 Kb fragment was excised and purified. The purified plasmid DNA was then dephosphorylated using calf intestinal alkaline phosphatase (Promega). 75 ng of dephosphorylated plasmid DNA was ligated with 60 ng and 120 ng of the ScaI-digested crtE-kan PCR product for 16 hours at 14°C using T4 DNA ligase (New England BioLabs). One μL of ligation mix was electroporated into 40 μL of *E. coli* ElectroMAX™ DH10B™ electrocompetent cells, which were then plated on LB media containing 10 μg/mL tetracycline, to which pSUP203 carries a resistance gene, and 25 μg/mL kanamycin. Plasmid DNA was isolated from cultures of individual colonies and digested with ScaI restriction enzyme to check insert size. 100 ng of plasmid DNA derived from a confirmed colony was electroporated into electrocompetent cells of the *E. coli* strain S17-1. This strain contains a

chromosomal copy of the trans-acting elements that mobilize oriT-containing plasmids during conjugation with a second bacterial strain. It also carries a gene conferring resistance to the antibiotics streptomycin and spectinomycin. The transformation reaction was plated on LB media with 10 μg/mL tetracycline, 25 μg/mL kanamycin, and 25 μg/mL streptomycin. Individual colonies were resuspended in about 20 μL of 10 mM Tris and heated for 10 minutes at 95°C to break open the bacterial cells. Two μL of the heated cells was used in a 25 μL PCR reaction using the CRTESCAF and CRTESCAR primers to confirm the presence of the crtE-kan insert. The PCR reaction mix contained 0.2 μM each primer, 1X GC Genomic PCR Buffer, 1.0 M GCMelt, 1.1 mM $Mg(OAc)_2$, 0.2 mM each dNTP, and 1X Advantage-GC Genomic Polymerase Mix. The PCR was conducted in a Perkin Elmer Geneamp 9600 and consisted of an initial denaturation at 94°C for 1 minute; 30 cycles of a 30 second denaturation at 94°C, a 1 minute annealing at 56°C, and a 4 minute extension at 72°C; followed by a final extension at 72°C for 5 minutes.

The pSUP203crtE-kan construct was introduced into *R. sphaeroides* strain 35053 through conjugation with the *E. coli* S17-1 strain carrying this vector. The S17-1 donor was grown in LB media with 25 μg/mL kanamycin and 25 μg/mL streptomycin at 37°C for 16 hours. A growing culture of *R. sphaeroides* strain 35053 was used to inoculate Sistrom's media using 1/5 to 1/10 dilutions, and the subcultures were grown at 30°C for about 20 hours. For both the S17-1crtE-kan and 35053 genotypes, cells were pelleted from 1.5 mL of culture. Pellets were resuspended and pelleted four times in either 1X Sistrom's salts for the 35053 cells or LB media for the S17-1 cells. The pellets were each resuspended in 1.5 mL of LB, and 200 μL of the S17-1 cells was combined with 1.3 mL of the 35053 cells. This mixture was pelleted, the supernatant removed, and the pellet resuspended in 20 μL of LB media. The resuspended cells were spotted onto an LB plate and incubated at 30°C for 7.5 hours. The cells were then scraped off the plate, resuspended in 1.5 mL of 1X Sistrom's salts, and plated (200 μL/plate) on Sistrom's media supplemented with 25 μg/mL kanamycin and 10 μg/mL of telluride (SisKTell). The telluride retards the growth of *E. coli* cells but is detoxified by *R. sphaeroides*. After 7 days, small black colonies were picked off the plates and streaked to fresh plates of the same media. After 6 days of growth, grayish colonies were patched to LB plates containing 25 μg/mL kanamycin (LBK25) and also to LB plates containing 0.75 μg/mL

tetracycline. Desirable double-crossover events, in which the crtE-kan gene was integrated and retained in the genome while the vector DNA was lost, exhibited kanamycin resistance but lacked tetracycline resistance. Colonies resulting from undesirable single-crossover events demonstrated both kanamycin and tetracycline resistance.

The mutants were confirmed using PCR and Southern hybridization as follows. Colonies that exhibited kanamycin resistance, lacked tetracycline resistance, and had a gray phenotype were screened by PCR for the crtE locus using the CRTESCAF and CRTESCAR primers as described above. To confirm that they were *R. sphaeroides* colonies with a truncated crtE gene rather than *E. coli* colonies carrying the vector, colonies were also screened using primers specific to the *R. sphaeroides* ppsR gene and the *E. coli* dxs gene. Individual colonies were resuspended in about 20 μL of 10 mM Tris, and heated for 10 minutes at 95°C to break open the bacterial cells. Two μL of the heated cells were used per 25 μL PCR reaction. The PCR reaction mix contained 0.2 μM each primer, 1X GC Genomic PCR Buffer, 1.0 M GCMelt, 1.1 mM $Mg(OAc)_2$, 0.2 mM each dNTP, and 1X Advantage-GC Genomic Polymerase Mix. The PCR was conducted in a Perkin Elmer Geneamp 9600 and consisted of an initial denaturation at 94°C for 1 minute; 8 cycles of a 30 second denaturation at 94°C, a 1 minute annealing at 55°C, and a 3.5 minute extension at 72°C; 22 cycles of a 30 second denaturation at 94°C, a 1 minute annealing at 61°C, and a 3.5 minute extension at 72°C; followed by a final extension at 72°C for 5 minutes. All suspected 35053crtE-kan colonies produced a crtE band the same size as the S17-1crtE-kan control. They all also produced a band of the expected size for the ppsR gene and did not produce a band for the *E. coli* dxs gene.

To further confirm the presence of double-crossover events, Southern hybridization was conducted on eight 35053crtE-kan colonies as well as *R. sphaeroides* strains 35053 and 17023. Sequence data for the photosynthetic operon of strain 17023 is available in Genbank and was used to determine restriction enzymes likely to have hybridization patterns that would distinguish mutants from non-mutants. Genomic DNA was isolated from each line using a Gentra Puregene DNA Isolation Kit (Gentra, Minneapolis, MN). Two μg of genomic DNA was used in digests with the restriction enzymes ApaI and XhoI. The digests were separated on a 0.8% TAE agarose gel, and the

DNA transferred to a nylon membrane. DIG-labeled molecular weight markers II and III (Roche) were also included on the gel/membrane. DIG-labeled probes of the crtE locus were synthesized using a PCR DIG Probe Synthesis Kit (Roche). After baking, membranes were prehybridized in EasyHyb Buffer (Roche) for at least 2 hours and hybridized overnight using 400 nL of a 0.5 DIG labeling reaction per mL of hybridization solution. Detection was conducted using a Wash and Block Buffer Set (Roche). Membranes were washed two times for 5-10 minutes each at room temperature in 2X SSC/0.1% SDS and two times for 15-20 minutes each at 68°C in 0.1X SSC/0.1% SDS. They were then covered with blocking buffer and placed on a shaker for an hour at room temperature. The blocking buffer was replaced with fresh blocking buffer containing 150 mU of AP conjugate per mL of buffer, and the membranes shaken at room temperature for an additional 30 minutes. Membranes were then washed twice for 15 minutes each at room temperature with washing buffer, followed by a five minute wash with detection buffer. The detection buffer was replaced with fresh detection buffer containing 20 µL of NBT/BCIP solution per mL of buffer. This was placed in the dark at room temperature with no shaking until color developed, after which the buffer was replaced with 10 mM Tris-1 mM EDTA solution.

In the ApaI digest, the mutant lines exhibited a band of about 850 bp larger than the strain 35053 control, which is the size difference expected from the insertion of the kanamycin gene product in the StuI/XhoI sites. For the XhoI digest, strain 35053 exhibited a band of about 700 bp, strain 17023 had a band of about 1100 bp, mutant 7C had a band of 1550 bp, and the remaining mutants had a band of 2050 bp. The reason for the size difference in the XhoI bands for the mutants was unclear, but mutant 7C was used in further studies due to its possession of the expected band size relative to strain 35053. The resulting *R. sphaeroides* mutant containing a crtE knockout was designated ATCC 35053/ΔcrtE(kan).

ATCC 35053/ΔcrtE

R. sphaeroides cells lacking crtE were made using sacB selection as follows. A truncated crtE gene was cloned into the vector pL01, which is a suicide vector in *R. sphaeroides*. The pL01 vector carries a kanamycin resistance gene, a *B. subtilis* sacB

gene, an oriT sequence, a ColEI replicon, and a multiple cloning site (Lenz *et al.*, *J. Bacteriol.*, 176(14):4385-93 (1994)). The pL01crtE plasmid was introduced into *R. sphaeroides* strain 35053 through conjugation with an *E. coli* donor. The kanamycin resistance gene was used to select for single-crossover events between the truncated crtE gene and the genomic crtE gene that resulted in incorporation of the pL01crtE DNA into the genome. The presence of the sacB gene on the vector allowed for subsequent selection for the loss of the vector DNA from the genome, as expression of this gene in the presence of sucrose is lethal to *E. coli* and to *R. sphaeroides* under certain growth conditions. A portion of the double-crossover events that led to loss of the sacB gene contained the truncated crtE allele. This method of gene knockout is useful because no residual antibiotic resistance gene is left in the genome.

A three-step PCR process was used to create a 249 bp in-frame deletion in the crtE gene. The crtE gene from *R. sphaeroides* strain 35053 was amplified by PCR using primers designed to introduce an SphI restriction site at the beginning of the amplified fragment and a SacI restriction site at the end of the amplified fragment. The sequences of the primers were as follows.

CRTESPHF 5'-CGTGGCATGCGTGTAAGAAAAAGTTGACA-CCTGTGGAGTC-3' (SEQ ID NO:172)

CRTESACR 5'- CTAAGAGCTCAGTTCGGGCTCGGTCTCGC-CTTTCAGGAAG -3' (SEQ ID NO:173)

The PCR reaction mix contained 0.2 μM each primer, 1X Genome Advantage reaction buffer, 1 M GCMelt, 1.1 mM $Mg(OAc)_2$, 0.2 mM each dNTP, 1X Genome Advantage Polymerase, and 1 ng of genomic DNA per μL of reaction mix. The PCR was conducted in a Perkin Elmer Geneamp 2400 and consisted of an initial denaturation at 94°C for 2 minutes; 32 cycles of a 30 second denaturation at 94°C, a 45 second annealing at 64°C, and a 3 minute extension at 72°C, followed by a final extension at 72°C for 7 minutes. 200 μL of PCR product was separated on a 1% TAE-agarose gel, and a 1.5 Kb fragment was excised and purified.

The second round of PCR consisted of two separate reactions: reaction A, which used primers CRTESPHF and CRTERI, and reaction B, which used primers CRTESACR and CRTEFI. The sequences of primers CRTEFI and CRTERI were as follows.

CRTEFI 5'-GAGAGCGAGAGCCAGATCAAGAAGSGGCTG-AAGGACATCC-3' (SEQ ID NO:174)

CRTERI 5'-GGATGTCCTTCAGCCSCTTCTTGATCTGGCT-CTCGCTCTC-3' (SEQ ID NO:175)

The 20 nucleotides on the 3' ends of this pair of primers are located near the center of the crtE gene, 249 bases apart from each other and facing towards the start (CRTERI) and end (CRTEFI) of the gene. The 20 bp on the 5' ends of these primers are the reverse complement of the 3' end of the other primer in the pair. PCR of the two separate reactions was conducted as in the first round, with the exception that 0.05 ng of first round product per μL of reaction mix was used as template. Also, the thermocycler program used a 2 minute initial denaturation at 94°C; eight cycles of a 30 second denaturation at 94°C, a 45 second annealing at 56°C, and a 3 minute extension at 72°C, followed by eight cycles of a 30 second denaturation at 94°C, a 45 second annealing at 60°C, and a 3 minute extension at 72°C; followed by 16 cycles of a 30 second denaturation at 94°C, a 45 second annealing at 64°C, and a 3 minute extension at 72°C; followed by a final extension at 72°C for 7 minutes. Both PCR products, about 590 and 650 bp in length, were separated on a 1% TAE-agarose gel, excised, and gel purified.

The third round of PCR used the same primers and reaction mixture as the first round of PCR with the exception that a mixture of 10 ng of each second round fragment was used as template rather than genomic DNA (200 μL reaction). The PCR program used was also the same as that used in the first round of PCR with the annealing time lengthened to 1.5 minutes. The 1.2 Kb third-round product was separated on a 1% TAE-agarose gel and purified. Three μg of purified DNA was digested with the restriction enzymes SacI and SphI. The digested DNA was cleaned using a QIAquick PCR Purification Kit and digested with the restriction enzyme StuI. StuI cut within the deleted

region and ensured that there was little or no remaining full-length product. The digestion mixture was again cleaned using a QIAquick PCR Purification Kit.

Three μg of the vector pL01 was digested with the restriction enzymes SphI and SacI. The enzymes were inactivated by heating to 65°C for 20 minutes, and the vector was dephosphorylated using shrimp alkaline phosphatase (Roche). The dephosphorylated vector DNA was gel purified on a 1% TAE-agarose gel.

Sixty-six ng of digested vector DNA was ligated with 80 ng of the digested third-round PCR product at 16°C for 16 hours using T4 DNA ligase (Roche). One μL of ligation mix was electroporated into 40 μL of *E. coli* ElectroMAX™ DH5α™ electrocompetent cells (Life Technologies), which were then plated on LB media containing 50 μg/mL kanamycin (LBK50). Plasmid DNA was isolated from cultures of individual colonies and digested with the restriction enzyme SacI and with a mixture of SphI and SacI to confirm correct vector structure.

One μL of plasmid DNA was used to transform electrocompetent cells of the previously described *E. coli* strain S17-1. The electroporated cells were plated on LB media containing 25 μg/mL of kanamycin, 25 μg/mL of streptomycin, and 25 μg/mL of spectinomycin (LBKSMST). Single colonies were used to start cultures for plasmid DNA isolation and used in conjugation. These colonies were also plated on LB media containing 5% sucrose and 25 μg/mL of kanamycin to ensure that the sacB gene was still functional. Only colonies which exhibited lethality on the sucrose media were used in conjugation. The presence of the correct insert size was confirmed by digestion of plasmid DNA with the restriction enzymes SacI and SphI.

Growing cultures of *R. sphaeroides* strain 35053 were sub-cultured, using 1/5 and 1/10 volumes of inoculum, in 5 mL Sistrom's media supplemented with 20% LB and grown at 30°C for 12 hours. The S17-1 donor colonies were grown in LBKSMST media at 37°C for 12 hours. 1.5- 3.0 mL of each culture was pelleted, and the pellets were washed four times with LB media. Relative pellet size was estimated and about 2 volumes of 35053 cells were used to 1 volume of S17-1 cells. The cell mixture was pelleted, resuspended in 20 μL of LB media, spotted on an LB plate, and incubated at 30°C for 7- 15 hours. The cells were then scraped off the surface of the plate and

resuspended in 1.5 mL of Sistrom's salts. 200 μL of resuspended cells were plated on each of seven plates of SisKTell media.

Colonies that grew on the plates after about 10 days, representing proposed single-crossover events, were streaked to new plates of the same media. Upon growth, single colonies were streaked out on LBK25 media. Purified colonies were patched to Sistrom's media supplemented with 1X LB, 15% sucrose, 0.5% DMSO (v/v), and 25 μg/mL kanamycin (SisLBK15%SucDMSO). These were grown in an anaerobic chamber (Becton Dickinson, Sparks, MD) at 30°C for 5 days to check for lethality of the sacB gene in the proposed single-crossover events. Concurrently, the cultures were patched to SisLB media containing 15% sucrose and 0.5% DMSO (v/v) without kanamycin (SisLB15%SucDMSO). Several of the cultures exhibited both white and red colonies upon growth on this media. Whitish-gray colonies were purified from these cultures and tested by PCR to show that they contained the truncated crtE allele. These colonies were also screened using primers specific to the *R. sphaeroides* ppsR gene and the *E. coli* dxs gene as described above. Potential double crossovers were also streaked on LBK25 plates to confirm that they were now sensitive to kanamycin. The resulting *R. sphaeroides* mutant containing a crtE knockout was designated ATCC 35053/ΔcrtE.

Several discoveries were made using the sacB method to knockout nucleic acid sequenced within the *R. sphaeroides* genome. First, it was discovered that the cultures used in conjugations, particularly those of the recipient *R. sphaeroides* strain, should be in exponential growth. Second, it was discovered that when using the S17-1 strain as a vector donor, the use of telluride in the plating medium is unnecessary as this strain is a proline auxotroph and will not grow on Sistrom's media without LB supplementation. Third, it was discovered that potential single crossovers should be screened using two separate PCR reactions. The first reaction should use a primer within the gene of interest together with a primer homologous to upstream sequence. The second reaction should use a primer within the gene of interest together with a primer homologous to downstream sequence. One of these two reactions should produce a truncated fragment. Fourth, it was discovered that single crossovers that have been confirmed to have sacB lethality can be grown aerobically in Sistrom's media for 2 days and then plated on SisLB15%SucDMSO media. The volume plated varies depending on the rate of growth

of the strain, but is about one μL or less for strain 35053. This is then grown anaerobically for about 5 days. Fifth, it was discovered that the sacB gene may not completely kill cells with the gene, so there may be a background level of very small colonies. The desired double-crossover colonies, however, are typically larger. These colonies should be purified and screened by PCR to identify whether they contain the truncated or full-length allele. Sixth, it was discovered that using one primer homologous to sequence upstream of the knockout gene and one primer homologous to sequence downstream of the gene is useful in confirming the correct location of the insertion event in addition to determining the allele that is present.

ATCC 35053/ΔppsR(strep)

R. sphaeroides cells lacking PPSR were made by inserting a spectinomycin/streptomycin resistance gene into the ppsR sequence as follows. To PCR amplify the ppsR gene from *R. sphaeroides* strain 17023, the following primers were designed based on published sequence (GenBank Accession Number L19596).

PPSRF2 5'-AGTCAGTACTAACTGGTGAAGACGCTGAAG-3' (SEQ ID NO:176)
PPSRR2 5'-GATCAGTACTGTGAACGAATACGATACGCA-3' (SEQ ID NO:177)

Each primer contained a ScaI restriction site. The ppsR gene was amplified using following reaction mix and PCR amplification program.

Reaction Mix	
pfu 10X buffer	10 μL
DMSO	5 μL
dNTP mix (10 mM)	8 μL
PPSRF2 (50 μM)	2 μL
PPSRR2 (50 μM)	2 μL
Genomic DNA (50 ng/μL)	2 μL
pfu enzyme (2.5 U/μL)	2 μL
DI water	69 μL
Total:	100 μL

Program		
94°C	5 minutes	
8 cycles of:		
	94°C	45 seconds
	54°C	45 seconds
	72°C	3 minutes
25 cycles of:		
	94°C	45 seconds
	61°C	45 seconds
	72°C	3 minutes
72°C	10 minutes	
4°C	Until used further	

The PCR product was separated on a 0.8% TAE agarose gel, and a band of about 1.8 Kb was cut and gel isolated using Qiagen Gel Isolation kit (Qiagen, Valencia, CA). The gel isolated DNA was digested with ScaI (New England BioLabs, Beverly, MA) for 5 hours. The digested DNA was column purified using Qiagen Gel Isolation kit. The cut DNA was ligated into vector pSUP203 that was also digested with ScaI enzyme.

2.3 μg of pSUP203 plasmid DNA was digested for 4 hours at 37°C with ScaI restriction enzyme. The digested DNA was separated on a 1% TAE agarose gel. A 7.6 Kb fragment was excised and purified. The purified plasmid DNA was then dephosphorylated using calf intestinal phosphatase (New England Biolabs). 100 ng of dephosphorylated plasmid DNA was ligated with 200 ng of the ScaI-digested PpsR DNA for 16 hours at 14°C using T4 DNA ligase (New England BioLabs). One μL of ligation mix was electroporated into 40 μL of *E. coli* ElectroMAX™ DH5α™ (Life Technologies, Gaithersburg, MD) electrocompetent cells, which were then recovered in 1 mL of SOC media for one hour at 37°C and plated on LB media containing 15 μg/mL tetracycline. Plasmid DNA was isolated from 8 individual colonies using Qiagen spin Mini prep kit and digested with ScaI restriction enzyme to check insert size. Four of the colonies had a correct insert. 1.5 μg of the plasmid DNA obtained from confirmed colony was digested with XhoI restriction enzyme (New England BioLabs, Beverly, MA). This enzyme has a single restriction site in the open reading frame of ppsR gene. A linear DNA band of about 8.4 Kb was gel isolated using a Qiagen Gel isolation kit. A spectinomycin/streptomycin resistance omega cassette was obtained by digesting plasmid pUI1638 (Obtained from Dr. Samuel Kaplan's laboratory) with XhoI enzyme. The digest was separated on a 0.8% TAE agarose gel, and a DNA band of about 2.1 Kb was gel isolated. This DNA which encoded for spectinomycin/streptomycin resistance gene was ligated to pSUP203/PpsR, which was also restricted with XhoI enzyme. One μL of ligation mix was electroporated into 40 μL of *E. coli* ElectroMAX™ DH5α™ (Life Technologies, Gaithersburg, MD) electrocompetent cells, which were then recovered in 1 mL of SOC media for one hour at 37°C and plated on LB media with 15 μg/mL tetracycline, 25 μg/mL spectionomycin, and 25 μg/mL streptomycin. Plasmid DNA was isolated from 10 individual colonies using Qiagen spin Mini prep kit and digested separately with ScaI and XhoI restriction enzyme to check insert size. Five of the

colonies had a correct insert. 100 ng of plasmid DNA from a confirmed colony was electroporated into electrocompetent cells of the *E. coli* strain SM10. This strain contains a chromosomal copy of the trans-acting elements that mobilize oriT-containing plasmids during conjugation with a second bacterial strain. It also carries a gene conferring resistance to the antibiotic kanamycin. The transformation reaction was recovered in 1 mL of SOC media for one hour and plated on LB media with 10 μg/mL tetracycline, 25 μg/mL kanamycin, 25 μg/mL of streptomycin, and 25 μg/mL spectinomycin.

The pSUP203/ppsR-SM-ST construct was conjugated from the *E. coli* SM10 host into *R. sphaeroides* strain 35053. The SM10 donor was grown in LB media with 25 μg/mL kanamycin, 25 μg/mL streptomycin, and 25 μg/mL spectinomycin at 37°C for 16 hours. A growing culture of *R. sphaeroides* strain 35053 was used to inoculate Sistrom's media in 1/5 to 1/10 dilutions. These cultures were grown for about 20 hours. Cells were pelleted for 1.5 mL of culture of both the SM10 pSUP203/PpsR-SM-ST and 35053 genotypes. Pellets were washed four times in Sistrom's media without vitamins and glucose. The pellets were each resuspended in 1.5 mL of Sistrom's media without vitamins and glucose. 200 μL of the SM10 pSUP203/PpsR-SM-ST cells were combined with 1.3 mL of the 35053 cells. This mixture was pelleted, the supernatant was removed, and the pellet was resuspended in 20 μL of LB media. The resuspended cells were spotted onto a LB plate that was then incubated at 30°C for 7 hours. The cells were then scrapped off the LB plate, resuspended in 1.5 mL of 1X Sistrom's media without vitamins and glucose, and plated (200 μL/plate) on Sistrom's media supplemented with 25 μg/mL spectinomycin, 25 μg/mL streptomycin, and 10 μg/mL of telluride. The telluride retards the growth of *E. coli* cells but is detoxified by *R. sphaeroides*. After 7-10 days, small black colonies were picked off the plates and streaked to fresh plates of the same media. After 6 days of growth, colonies were patched to LB plates containing 25 μg/mL spectinomycin and 25 μg/mL streptomycin (LBSMST25), and also to LB plates containing 0.75 μg/mL tetracycline. Desirable double-crossover events, in which the PpsR-SM-ST gene is retained in the genome and the vector DNA is lost, would have spectinomycin/streptomycin resistance but lack tetracycline resistance. Colonies resulting from undesirable single-crossover events would demonstrate resistance to all of these antibiotic markers.

Colonies that exhibited only spectinomycin/streptomycin resistance and displayed deep red color were confirmed for double-crossover by Southern hybridization. Southern hybridization was conducted on nineteen potential 35053/PpsR-SM-ST colonies in addition to 35053 and *R. sphaeroides* strain 17023. Sequence data for the photosynthetic operon of 17023 is available in Genbank and was used to determine restriction enzymes likely to have hybridization patterns that would distinguish mutants from non-mutants. Genomic DNA was isolated from each line using a Gentra Puregene DNA Isolation Kit (Gentra, Minneapolis, MN). 2 μg of genomic DNA was used in digests using the restriction enzymes NcoI, ApaI, and XmaI in separate reactions. The digests were separated on a 1% TAE agarose gel, and the DNA was transferred to nylon membrane (Roche Molecular Biochemicals, Indianapolis, IN). DIG-labeled molecular weight markers II and III (Roche) were also included on the gel/membrane. DIG-labeled probes of the PpsR locus were made using a PCR DIG Probe Synthesis Kit (Roche). After baking, membranes were prehybridized in EasyHyb Buffer (Roche) for at least 2 hours and hybridized overnight using 400 nL of a 0.5 DIG labeling per mL of hybridization solution. Detection was done using a Roche Wash and Block Buffer Set (Roche). Membranes were washed two times for 5-10 minutes at room temperature in 2X SSC/0.1% SDS and two times for 15-20 minutes at 68°C in 0.1X SSC/0.1% SDS. They were then covered with blocking buffer and placed on a shaker for an hour at room temperature. The blocking buffer was replaced with fresh blocking buffer containing 150 mU of AP conjugate per mL of buffer, and the membranes shaken at room temperature for an additional 30 minutes. Membranes were then washed twice for 15 minutes at room temperature with washing buffer, followed by a five minutes wash with detection buffer. The detection buffer was replaced with fresh detection buffer containing 20 μL of NBT/BCIP solution per mL of buffer. This was placed in the dark at room temperature with no shaking until sufficient color was developed.

In the NcoI digest, the lanes of colony 9 and 10 exhibited a band about 2 Kb larger than the 35053 control, which is the size difference expected from the insertion of the spectinomycin/streptomycin resistance cassette into the XhoI site For the XmaI digest, 35053 exhibited a single band about 5.5 Kb, while colonies 9, 10, and 5 exhibited two bands whose summed size was about 2 Kb higher than that of 35053. Two bands were

observed in colony 9, 10, and 5 because a XmaI was introduced along with the spectinomycin/streptomycin resistance cassette. For ApaI digest, the control 35053 sample exhibited two bands since ppsR gene harbors an ApaI site. Each of these bands was about 2.3 Kb in size. Colony 9, 10, and 5 exhibited three bands, whose summed size was about 2 Kb higher band that of 35053. An extra band was observed in colonies 9, 10, and 5 because an ApaI site was introduced along with the spectinomycin/streptomycin resistance cassette.

The resulting *R. sphaeroides* mutant containing the ppsR knockout was designated ATCC 35053/ΔppsR(strep).

ATCC 35053/ΔppsR

R. sphaeroides cells lacking ppsR were made using sacB selection as follows. A three-step PCR process was used to create a 255 bp in-frame deletion in the PpsR gene, so that there would be no residual antibiotic resistance gene in the genome. The PpsR gene from *R. sphaeroides* strain 35053 was amplified by PCR using primers designed to introduce an SacI restriction site at the beginning of the amplified fragment and a SphI restriction site at the end of the amplified fragment. The sequences of the primers were as follows.

PPSRSACF2 5'-GTCAAATGAGCTCCAAACTGGTGAAGA-CGCTGAAGGACAT-3' (SEQ ID NO:178)

PPSRSPHR 5'-CAGTCGGGCATGCGTCCATTTCAGTTGAC-ATACTTCTGTG-3' (SEQ ID NO:179)

The following PCR mix program was used to amplify the PpsR gene.

Reaction Mix	
pfu 10X buffer	10 µL
DMSO	5 µL
dNTP mix (10 mM)	3 µL
PPSRSACF2 (100 µM)	1 µL
PPSRSPHR (100 µM)	1 µL
Genomic DNA (50 ng/µL)	2 µL

Program	
94°C	2 minutes
8 cycles of:	
94°C	30 seconds
58°C	45 seconds
72°C	3 minutes
25 cycles of:	

pfu enzyme (2.5 U/μL)	2 μL		94°C 30 seconds
DI water	76 μL		64°C 45 seconds
			72°C 3 minutes
Total:	100 μL	72°C	7 minutes
		4°C	Until used further

100 μL of PCR product was separated on a 1% TAE agarose gel, and a fragment about 1.8 Kb was excised and purified using Qiagen Gel isolation kit.

The second round of PCR consisted of two separate reactions: reaction A, which used primers PPSRSACF2 and PPSRMIDR, and reaction B, which used primers PPSRSPHR and PPSRMIDF. The sequences of primers PPSRMIDF and PPSRMIDR were as follows.

PPSRMIDF 5'-CTCTTGCTCGGCGGCGTGCGGCTCTATCA-
CGAGGGGGTGGA-3' (SEQ ID NO:180)

PPSRMIDR 5'-TCCACCCCCTCGTGATAGAGCCGCACGCC-
GCCGAGCAAGAG-3' (SEQ ID NO:181)

The 20 nucleotides on the 3' ends of this pair of primers are located near the center of the ppsR gene, 255 bases apart from each other, and facing towards the start (PPSRMIDR) and end (PPSRMIDF) of the gene. The 20 bp on the 5' ends of these primers are the reverse complement of the 3' end of the other primer in the pair. The following reaction mix and program were used to conduct these PCR.

Reaction Mix A	
pfu 10X buffer	10 μL
DMSO	5 μL
dNTP mix (10 mM)	3 μL
PPSRSACF2 (100 μM)	1 μL
PPSRMIDR (100 μM)	1 μL
DNA from first round (10 ng/μL)	1 μL
pfu enzyme (2.5 U/μL)	2 μL
DI water	77 μL
Total:	100 μL

Program	
94°C	2 minutes
8 cycles of:	
94°C	30 seconds
58°C	45 seconds
72°C	3 minutes
25 cycles of:	
94°C	30 seconds
64°C	45 seconds
72°C	3 minutes
72°C	7 minutes
4°C	Until further use

Reaction Mix B	
pfu 10X buffer	10 µL
DMSO	5 µL
dNTP mix (10 mM)	2 µL
PPSRSPHR (100 µM)	1 µL
PPSRMIDF (100 µM)	1 µL
DNA from first round (5ng/µL)	1 µL
pfu enzyme (2.5 U/µL)	2 µL
DI water	78 µL
Total:	100 µL

Program	
94°C	2 minutes
8 cycles of:	
94°C	30 seconds
58°C	45 seconds
72°C	3 minutes
25 cycles of:	
94°C	30 seconds
64°C	45 seconds
72°C	3 minutes
72°C	7 minutes
4°C	Until further use

Both PCR products, about 800-700 bp in length, were separated on a 1% TAE agarose gel, excised, and gel purified using a Qiagen gel isolation kit.

The third round of PCR used primers PPSRSACF2 and PPSRSPHR but used both fragments derived in the second round of PCR as template. The PCR mixture used was the same as in the first round of PCR except that equal molar amounts of the round 2 fragments were used as template. The PCR program used was also the same as that used in the first round of PCR, with the annealing time lengthened to 1.5 minutes. The 1.5 Kb third-round product was separated on a 1% TAE agarose gel and purified using Qiagen gel isolation kit. The purified DNA was digested overnight at 37°C with the restriction enzymes SacI and SphI.

Three µg of the vector pL01 was digested with the restriction enzymes SphI and SacI at 37°C for 16 hours. The enzymes were inactivated by heating to 65°C for 20 minutes. Dephosphorylation of the vector was achieved by adding 4.7 µL of shrimp alkaline phosphatase 10X buffer (Roche) and 2 µL of shrimp alkaline phosphatase to the inactivated digest. This mixture was heated at 37°C for 10 minutes and then 65°C for 15 minutes. The dephosphorylated vector DNA was then gel purified on a 1.0% TAE agarose gel.

98 ng of vector DNA was ligated with 210 ng of the digested third round PCR at 14°C for 14 hours using T4 DNA ligase (Roche). One µL of ligation mix was electroporated into 40 µL of *E. coli* ElectroMAX™ DH5α™ electrocompetent cells (Life Technologies), which were then recovered in 1 mL of SOC media for one hour and plated

on LB media with 25 μg/mL kanamycin (LBK25). Plasmid DNA was isolated from eight individual colonies. Plasmid DNA was checked for correct insert with a PCR screen using the PCR protocol from first round.

One μL of plasmid DNA was used to transform electrocompetent cells of *E. coli* strain S17-1. The electroporated cells were recovered in 1 mL of SOC media for one hour and plated on LB media with 25 μg/mL of kanamycin, 25 μg/mL of streptomycin, and 25 μg/mL of spectinomycin (LBKSMST). Single colonies were used to start cultures for plasmid DNA isolation and used in conjugation. These colonies were also plated on LB media containing 5% or 15% sucrose, and 25 μg/mL of kanamycin to ensure that the sacB gene was still functional. Only colonies that showed lethality on the sucrose media were used in conjugation. The presence of the correct insert size was confirmed by colony PCR.

Growing cultures of *R. sphaeroides* strain 35053 were subcultured, using 1/4 and 1/8 volumes of inoculum, in 5 mL Sistrom's media supplemented with 20% LB and grown at 30°C for 9 hours. The S17-1 donor colonies were grown in LBKSMST media at 37°C for 16 hours. 3.0 mL of 35053 and 0.5 mL of S17-1 donor cells were centrifuged and washed four times in Sistrom's media without glucose. Each cell pellet was resuspended into 20 μL LB, and the S17-1 donor suspension was mixed with 35053. The mixture was then spotted on LB, which was incubated at 30°C for 14-16 hours. The cells were then scraped off the surface of the plate and resuspended in 1.5 mL of Sistrom's salts. 200 μL of resuspended cells were plated on each of the seven Sistrom's media plates that were supplemented with 25 μg/mL of kanamycin.

Colonies that grew on the plates after about 10-14 days, representing proposed single crossover events, were streaked to new plates of the same media. Upon growth, single colonies were transferred to LBK25 media. These cultures were grown for 36 to 48 hours in Sistrom's media supplemented with 20% LB and no kanamycin at 30°C. 0.1 μL and 5 μL of this culture was plated on LB media that was supplemented with Sistrom's salts and 15% sucrose. The plates were placed in an anaerobic chamber (Becton Dickinson, Sparks, MD), and the chamber was placed in a 30°C incubator. After 4-5 days, several colonies showed up on the plates, indicating the occurrence of double-crossover events. Four colonies from each single-crossover strain were purified by

streaking on LB agar plates. Single colonies of double-crossover strains were screen by PCR for integration of truncated version of the ppsR gene into the chromosome. For screening, the following primers were used, which were located upstream and downstream of the PpsR gene. The use of upstream and downstream primer confirms both the locus of integration as well as truncation of PpsR gene.

PPSRUPF 5'-GAGCAGCACACTCTGGGAGC-3' (SEQ ID NO:182)
PPSRDNR 5'-CCACACAGGTAGGACACCCAC-3' (SEQ ID NO:183)

The following reaction mix and PCR program was used.

Reaction Mix		Program	
Taq Mg+ 10X buffer	2.5 µL	94°C	2 minutes
DMSO	1.25 µL	29 cycles of:	
dNTP mix (10 mM)	0.5 µL	94°C	30 seconds
PPSRUPF (100 µM)	0.125 µL	61°C	45 seconds
PPSRDNR (100 µM)	0.125 µL	72°C	3 minutes
Cell boil mix	2 µL	72°C	7 minutes
Taq enzyme (5 U/µL)	0.2 µL	4°C	Until further use
DI water	18.3 µL		
Total:	25 µL		

The cell boil mix was prepared by resuspending a single colony in 20-25 µL of water. The suspension was heated at 95°C for 10 minutes in a PCR machine. The tube was given a quick spin to pellet the solids.

The colonies that exhibited the truncated version of the PpsR gene were further tested for kanamycin sensitivity by streaking them on LB plates that were supplemented with 25 µg/mL of kanamycin. Also, these colonies were PCR screened for the kanamycin resistance gene.

The resulting *R. sphaeroides* mutant containing the ppsR knockout was designated ATCC 35053/ΔppsR.

ATCC 35053/ΔccoN

R. sphaeroides cells lacking ccoN were made using sacB selection as follows. A mutant of *R. sphaeroides* strain 2.4.1 having a 546 bp deletion in the ccoN gene (*R. sphaeroides* 2.4.1/ΔccoN) was obtained from the laboratory of Samuel Kaplan at the University of Texas (Oh and Kaplan, *Biochemistry*, 38:2688-2696 (1999)). The mutated ccoN locus of this strain was amplified by PCR and cloned into pL01. This plasmid was transformed into *E. coli* strain S17-1. The S17-1 strain was conjugated with *R. sphaeroides* strain 35053, and colonies were identified in which the truncated locus had replaced the native ccoN gene.

The truncated ccoN gene from *R. sphaeroides* 2.4.1/ΔccoN was amplified by PCR using primers designed to introduce a SacI restriction site at the beginning of the amplified fragment and a SphI restriction site at the end of the amplified fragment. The sequences of the primers were as follows.

CCONSACF 5'-TCAGAGCTCGTGTGATCGAATGGGGCTTT-GTTCCTTGATG-3' (SEQ ID NO:184)

CCONSPHR 5'-GAAGCATGCAGGTGATCGACGTGCCACTC-GTCCGAATAG-3' (SEQ ID NO:185)

The PCR reaction mix contained 0.2 μM each primer, 1X Native Pfu reaction buffer, 0.2 mM each dNTP, 5% DMSO, and 10 units of Pfu DNA polymerase in a 200 μL reaction. Three μL of the glycerol stock was diluted in 20 μL of 10 mM Tris and heated at 94°C for 10 minutes, after which 4 μL was added to the PCR reaction. The PCR was conducted in a MJ Research PT100 and consisted of an initial denaturation at 94°C for 2 minutes; 32 cycles of a 30 second denaturation at 94°C, a 1 minute annealing at 66°C, and a 4 minute extension at 72°C, followed by a final extension at 72°C for 7 minutes. The PCR product was separated on a 1% TAE-agarose gel, and a 1.6 Kb fragment was excised and purified. Three μg of purified PCR product was digested with SacI restriction enzyme and separated on a 1% TAE gel. A 1.4 Kb band was excised and purified. A SacI restriction site exists about 200 bp from the CCONSPHR end of the original PCR product.

Three μg of the vector pL01 was digested with the restriction enzyme SacI. The enzyme was inactivated by heating to 65°C for 20 minutes, and the digested vector was dephosphorylated using shrimp alkaline phosphatase. The dephosphorylated vector DNA was gel purified on a 1% TAE-agarose gel.

50 ng of digested vector DNA was ligated with 65 ng of the digested ccoN PCR product at 16°C for 16 hours using T4 DNA ligase (Roche). One μL of ligation mix was electroporated into 40 μL of *E. coli* Electromax™ DH5α™ electrocompetent cells, which were then plated on LBK media. Plasmid DNA was isolated from cultures of individual colonies and digested with the restriction enzyme SacI to confirm correct insert size.

The *E. coli* strain S17-1 contains a chromosomal copy of the trans-acting elements that mobilize oriT-containing plasmids during conjugation with a second bacterial strain. It also carries genes conferring resistance to the antibiotics streptomycin and spectinomycin. In addition, S17-1 is a proline auxotroph and will not grow on unsupplemented Sistrom's media. One μL of DNA of the truncated ccoN construct was used to transform electrocompetent cells of *E. coli* strain S17-1. The electroporation was plated on LBKSMST. Single colonies were used to start cultures for plasmid DNA isolation and used in conjugation. These colonies were also plated on LB media containing 5% sucrose and 25 μg/mL of kanamycin to ensure that the sacB gene was still functional. Only colonies that exhibited lethality on the sucrose media were used in conjugation. The presence of the correct insert size was confirmed by digestion of plasmid DNA with the restriction enzyme SacI.

Growing cultures of *R. sphaeroides* strain 35053 were subcultured in Sistrom's media supplemented with 20% LB to ensure that they were in exponential growth. The S17-1 donor colonies were grown in LBKSMST media at 37°C overnight or subcultured from growing colonies. 2-4 mL of each culture was centrifuged, and the pellets were washed four times in LB media. Relative pellet size was estimated, and about 2 volumes of 35053 cells were used to 1 volume of S17-1 cells. The cell mixture was then pelleted, resuspended in 20 μL of LB media, and spotted on an LB plate. This plate was incubated at 30°C for 7- 15 hours. The cells were then scraped off the surface of the plate and resuspended in 1.2 mL of Sistrom's salts. 200 μL of resuspended cells were plated on each of six plates of Sistrom's media containing 25 μg/mL of kanamycin (SisK).

Colonies that grew on the plates after about 10 days, representing potential single-crossover events, were streaked to new plates of SisK media. Upon growth, single colonies were transferred to LBK media. Purified colonies were streaked to Sistrom's media supplemented with 1X LB, 15% sucrose, 0.5% DMSO (v/v), and 25 μg/mL kanamycin (SisLBK15%SucDMSO). These were grown in an anaerobic chamber (Becton Dickinson, Sparks, MD) at 30°C for 5 days to check for lethality of the sacB gene in the single-crossover events. The purified colonies were also screened in two separate PCR reactions. The first reaction used a primer within the gene of interest (CCONR) together with a primer homologous to upstream sequence (CCONUPF2), and the second reaction used a primer within the gene of interest (CCONSACF) together with a primer homologous to downstream sequence (CCONDNR2). Single-crossover events exhibited a truncated fragment in one of the two reactions, depending on whether the crossover occurred upstream or downstream of the deletion. The primer sequences were as follows.

CCONUPF2 5'-CTCACAACCTCCAACCGATG-3' (SEQ ID NO:186)
CCONR 5'-CGATGGTGACCACGAAGAAG-3' (SEQ ID NO:94)
CCONDNR2 5'-CGTAACGCTCGGTCTCGTC-3' (SEQ ID NO:129)

Single-crossover colonies were grown in Sistrom's media supplemented with 20% LB. After 2 days of growth, 0.1-1 μL of the cultures was plated on Sistrom's media supplemented with 1X LB, 0.5% DMSO (v/v), and 15% sucrose (SisLB15%SucDMSO). These cultures were grown anaerobically for about 5 days. The sacB gene did not always completely kill cells with the gene, so there was often a background level of very small colonies. The larger colonies, which represented double-crossover events, were purified on LB media and screened by PCR to identify whether they contained the truncated or full-length allele. The CCONUPF2 and CCONDNR2 primers were used in this PCR screen to ensure that the truncated gene also was inserted in the correct location in the genome. Potential double-crossovers were also streaked on LBK plates to confirm that they were now sensitive to kanamycin.

The resulting *R. sphaeroides* mutant containing the ccoN knockout was designated ATCC 35053/ΔccoN.

ATCC 35053/ΔcrtE/ΔccoN

R. sphaeroides cells lacking crtE and ccoN were made as follows. The wildtype ccoN allele of a crtE knockout mutant (ATCC 35053/ΔcrtE) was replaced with a truncated ccoN allele as described above. Double-crossover colonies having the truncated ccoN allele were then re-screened by PCR for the crtE and ccoN loci. These colonies were plated on LBK25 and screened by PCR to confirm the loss of the vector from the genome. The resulting *R. sphaeroides* mutant containing the crtE knockout and ccoN knockout was designated ATCC 35053/ΔcrtE/ΔccoN.

ATCC 35053/ΔcrtE/ΔppsR/ΔccoN

R. sphaeroides cells lacking crtE, ppsR, and ccoN were made as follows. The wildtype ppsR allele of a crtE/ccoN knockout mutant (ATCC 35053/ΔcrtE/ΔccoN) was replaced with a truncated ppsR allele as described above with the following exceptions. After conjugation on an LB plate, the conjugated cells were plated on Sistrom's media containing 25 μg/mL of kanamycin and 0.5% DMSO (SisKDMSO) rather than on SisK. After purification on SisKDMSO and LBKDMSO, single-crossovers were grown aerobically in Sistrom's media supplemented with 1X LB and 0.5% DMSO. After 2 days of growth, the cultures were plated on Sistrom's media supplemented with 1X LB, 15% sucrose, and 0.5% DMSO, and grown anaerobically for 5 days. Potential double-crossover colonies were purified on LBDMSO and screened by PCR using the PPSRUPF and PPSRDNR primers. Colonies having the truncated ppsR allele were then rescreened by PCR for the crtE, ppsR, and ccoN loci. These colonies were also plated on LBKDMSO and screened by PCR to confirm the loss of the vector from the genome. The resulting *R. sphaeroides* mutant containing the crtE knockout, ppsR knockout, and ccoN knockout was designated ATCC 35053/ΔcrtE/ΔppsR/ΔccoN.

Example 10 – Making recombinant microorganisms that overexpress a particular sequence while a containing knock-out

Any construct developed for the overexpression of genes are transferred to any of the background genotypes developed by gene knockout techniques. For example, the pMCS2tetP/Stdxs/Rsdds/EcUbiC or the pMCS2tetP/Stdxs/Rsdds/RsLytB construct is transferred into the *R. sphaeroides* ATCC 35053/ΔcrtE/ΔppsR/ΔccoN mutant cells to combine the productive effects of gene overexpression and engineering of gene regulation or carbon flow. The construct is transferred to the desired genotype by electroporation or conjugation. Conjugation of a plasmid into an *R. sphaeroides* strain follows the procedure described for the isolation of single-crossover events except that, since the efficiency of plasmid transfer is much higher than that of chromosomal integration, a 0.1-1 μL plating volume from the ~400 μL conjugation recovery is ample to obtain transformed colonies. Single colony PCR is used to check the integrity of the construct in the new background, and evaluations of the productivity of the new strain are made. Genes that are productive are integrated, in one or more copies, into appropriate regions of the chromosome of a productive strain along with or downstream of a highly-expressing promoter.

Example 11 - Three liter fermentations

Cultures of *R. sphaeroides* ATCC 35053 with various inserted genes or knockouts were grown in 5 mL culture tubes containing Sistrom's media with 4 g/L glucose. After 48 hours of growth at 30°C with 250 rpm shaking, the entire contents of the tube were used to inoculate a 300 mL baffled shake flask containing Sistrom's media with 4 g/L glucose. After incubation at 30°C for 48 hours, the entire contents of the flask were added to 2.7 L of Sistrom's media containing 40 g/L glucose in a B. Braun Biotech International Model Biostat B fermenter.

The fermenter was maintained at 30°C, and the cascade was set to maintain the dissolved oxygen (DO) at 40%. The air inflow was maintained at 1 vvm, and the pH was maintained at 7.3 with an automatic feed of 2N NH_4OH. Foaming was controlled by addition of Sigma Antifoam 289. Kanamycin to a concentration of 50 μg/mL was added to fermentations with strains containing the broad host range vector pBBRIMCS2 either

with or without an inserted gene. At 24 to 30 hours, when the agitation increase to maintain a DO of 40% had leveled off, the agitation and DO were decoupled, and the agitation was fixed at 240 rpm. The air inflow was lowered to 0.3 vvm. Kanamycin to 50 μg/mL was again added to fermentations containing the expression vector.

The fermentation samples for coenzyme Q10 and spheroidenone analysis were removed at 69 to 75 hours into the fermentation.

Example 12 - Three-hundred milliliter fermentations

Cultures of *R. sphaeroides* ATCC 35053 with various overexpressed genes or knockouts were grown in 5 mL culture tubes containing Sistrom's media with 4 g/L glucose. After 48 hours of growth at 30°C with 250 rpm shaking, the entire contents of the tube were used to inoculate a 300 mL baffled shake flask containing Sistrom's media with 4 g/L glucose. After incubation at 30°C for 48 hours, 30 mL of the flask were added to 270 mL of Sistrom's media containing 40 g/L glucose in a 500 mL Infors AG-CH-4103 fermenter.

The fermenter was maintained at 30°C, and the cascade was set to maintain the dissolved oxygen (DO) at 40%. The air inflow was maintained at 1 vvm, and the pH was maintained at 7.3 with an automatic feed of 2N NH_4OH. Foaming was controlled by addition of Sigma Antifoam 289. Kanamycin to a concentration of 50 μg/mL was added to fermentations with strains containing the broad host range vector pBBRIMCS2 either with or without an inserted gene. At 24 to 30 hours, when the agitation increase to maintain a DO of 40% had leveled off, the agitation and DO were decoupled, and the agitation was fixed at 400 rpm. The air inflow was lowered to 0.3 vvm. Kanamycin to 50 μg/mL was again added to fermentations containing the expression vector.

The fermentation samples for coenzyme Q10 and spheroidenone analysis were removed at 69 to 75 hours into the fermentation.

Example 13 - Analysis of Spheroidenone

At various times during the fermentation, 15 mL of fermentation volume was withdrawn. The volume of sample needed to obtain 5 mg of dry cell weight (DCW) was used for spheroidenone analysis. The sample was washed one time in water and

resuspended in an equal volume of water. The volume of sample calculated in step 1 was added to a 1.8 mL-microfuge tube and was centrifuged at 10,000 rpm for 3 minutes in an IEC MicroMax microfuge. The supernatant was removed, and the pellet was completely resuspended in 1.0 mL of Acetone:Methanol (7:2) and stored at room temperature away from light for 30 minutes. The sample was mixed once during this incubation. After incubation, the sample was centrifuged at 10,000 rpm for 3 minutes, and the extract (supernatant) collected. Samples were stored –20°C for analysis at a later time. The carotenoid extract was analyzed on a spectrophotometer scanning in the range of 350 nm to 800 nm, and the OD_{480} was recorded. The amount of carotenoid in mg/100 mL of culture was calculated using the following equation:

Spheroidenone (mg) / 100 mL culture = $((OD_{480} – (0.0816 * OD_{770})) * 0.484)$ / Vol. of sample from step 1

From mg of Spheroidenone/100 mL of culture, the amount of Spheroidenone/mg of dry cell weight (DCW) was calculated using the DCW number as the conversion factor. Care was taken to correct for any dilution factor required while the sample was scanned on the spectrophotometer.

Example 14 – Analyzing CoQ(10) levels produced via fermentation

100 mL of fermentation broth was removed once per day and placed in a tared 250 mL centrifuge bottle. The samples were centrifuged at 15,000 X g for 5 minutes, the supernatant was poured off, and the samples were resuspended in 50 mL cold water. The samples were centrifuged again at 15,000 X g for 5 minutes, and the supernatant was poured off. The wet weight of the biomass was determined, and the biomass was resuspended in 1.5 times its weight in water. The samples were stored covered with foil at -80°C before analysis.

Before analysis, the samples were warmed at 21°C for 15 minutes. 1.0 mL was withdrawn. Sodium dodecyl sulfate was added to a final concentration of 1.67 %. The samples were extracted with 14 mL of a hexane:ethanol (5:2) mixture. The samples were then evaporated to dryness and dissolved in 2 mL of a methanol:ethanol (9:2) mixture.

The samples were then analyzed on a Waters Nova-Pak C18 (3.9 x 150 mm: 4 Um) column with a PDA detector set from 200-300 nm. Resolution was at 1.2 nm with a maximum absorbance at 275 nm. The run time was 15 minutes, and the injection volume was 20 μL.

The dry weight of the samples were determined drying an aliquot at 105°C in an aluminum weighing pan for at least four hours.

Example 15 – Production of CoQ(10)

The following seven experiments measured the amount of CoQ(10) produced by the indicated microorganisms in a 3 liter scale fermentation.

In experiment 1, the following data were collected after 96 hours of fermentation:

Strain	Coenzyme Q10 (ppm) dry weight basis
ATCC 35053	2950
ATCC 35053/ΔcrtE	6508

These results demonstrated that the inactivation of crtE increased the production of CoQ(10).

In experiment 2, the following data were collected after 69 to 75 hours of fermentation:

Strain	Coenzyme Q10 (ppm) dry weight basis
ATCC 35053	1655
ATCC 35053/ΔppsR(strep)	3812

These results demonstrated that the inactivation of ppsR increased the production of CoQ(10).

In experiment 3, the following data were collected after 69 to 75 hours of fermentation:

Strain	Coenzyme Q10 (ppm) dry weight basis	Spheroidenone (ppm) dry weight basis
ATCC 35053	2951	1980
ATCC 35053/ΔccoN	3527	2959

These results demonstrated that the inactivation of ccoN increased the production of CoQ(10) and spheroidenone.

In experiment 4, the following data were collected after 69 to 75 hours of fermentation:

Strain	Coenzyme Q10 (ppm) dry weight basis
ATCC 35053/ΔcrtE	3255
ATCC 35053/ΔcrtE/ΔccoN isolate 8-7	7951

These results demonstrated that the inactivation of crtE and ccoN increased the production of CoQ(10) as compared to inactivating crtE only.

In experiment 5, the following data were collected after 69 to 75 hours of fermentation:

Strain	Coenzyme Q10 (ppm) dry weight basis
ATCC 35053/ΔcrtE	3545
ATCC 35053/ΔcrtE/ΔccoN isolate 111	4984
ATCC 35053/ΔcrtE/ΔppsR/ΔccoN	11,676

These results demonstrated that the inactivation of crtE and ccoN increased the production of CoQ(10) as compared to inactivating crtE only. In addition, these results demonstrated that the inactivation of crtE, ccoN, and ppsR increased the production of CoQ(10) as compared to inactivating only crtE and ccoN.

In experiment 6, the following data were collected after 69 to 75 hours of fermentation:

Strain	Coenzyme Q10 (ppm) dry weight basis
ATCC 35053/ΔcrtE	3833
ATCC 35053/ΔcrtE/pMCS2tetP/Stdxs	4928
ATCC 35053/ΔcrtE/pMCS2glnP/Stdxs	5508
ATCC 35053/ΔcrtE/pMCS2tetP/Stdds	4652

These results demonstrated that the inactivation of crtE together with the addition of Stdxs increased the production of CoQ(10) as compared to inactivating crtE only. In addition, these results demonstrated that the use of the gln promoter with Stdxs resulted in more production of CoQ(10) when compared to the use of the tet promoter with Stdxs. Further, these results demonstrated that the inactivation of crtE together with the addition of Stdds increased the production of CoQ(10) as compared to inactivating crtE only.

In experiment 7, the following data were collected after 69 to 75 hours of fermentation:

Strain	CoQ(10) (ppm) dry weight basis
ATCC 35053/pMCS2tetP	3909
ATCC 35053/pMCS2tetP/Stdxs/Rsdds	5387
ATCC 35053/pMCS2tetP/Stdxs/Rsdds/RsLytB	5962
ATCC 35053/pMCS2tetP/Stdxs/Rsdds/EcUbiC	6439

These results demonstrated that the addition of Stdxs and Rsdds increased the production of CoQ(10) as compared to adding vector only. In addition, these results demonstrated that the addition of either RsLytB or EcUbiC together with the addition of Stdxs and Rsdds increased the production of CoQ(10) as compared to adding only Stdxs and Rsdds.

The following four experiments measured the amount of CoQ(10) produced by the indicated microorganisms in a 300 mL scale fermentation.

In experiment 1, the following data were collected after 69 to 75 hours of fermentation:

Strain	CoQ(10) (ppm) dry weight basis
ATCC 35053/pMCS2tetP	5250
ATCC 35053/pMCS2tetP/Stdxs	5758
ATCC 35053/pMCS2tetP/Rsdds	6944
ATCC 35053/pMCS2tetP/Stdxs/Rsdds	6875
ATCC 35053/pMCS2tetP/Stdxs/Rsdds/EcUbiC	7808

These results demonstrated that the addition of either Stdxs or Rsdds increased the production of CoQ(10) as compared to adding vector only. In addition, these results demonstrated that the addition of Stdxs, Rsdds, and EcUbiC increased the production of CoQ(10) as compared to adding only Stdxs and Rsdds.

In experiment 2, the following data were collected after 69 to 75 hours of fermentation:

Strain	CoQ(10) (ppm) dry weight basis
ATCC 35053/pMCS2tetP	5483
ATCC 35053/pMCS2tetP/EcubiC	6360
ATCC 35053/pMCS2tetP/RsLytB	5976
ATCC 35053/pMCS2tetP/Stdxs/Rsdds/RsLytB	6751

These results demonstrated that the addition of either EcUbiC or RsLytB increased the production of CoQ(10) as compared to adding vector only. In addition, these results demonstrated that the addition of Stdxs, Rsdds, and RsLytB increased the production of CoQ(10) as compared to adding only RsLytB.

In experiment 3, the following data were collected after 69 to 75 hours of fermentation:

Strain	CoQ(10) (ppm) dry weight basis
ATCC 35053/pMCS2tetP	5072
ATCC 35053/pMCS2tetP/Stdxs/Rsdds/RsLytB	8050

These results demonstrated that the addition of Stdxs, Rsdds, and RsLytB increased the production of CoQ(10) as compared to adding vector only.

In experiment 4, the following data were collected after 69 to 75 hours of fermentation:

Strain	Coenzyme Q10 (ppm) dry weight basis
ATCC 35053/pMCS2tetP	4503
ATCC 35053/pMCS2tetP/Stdxs/Rsdds	8833

These results demonstrated that the addition of Stdxs and Rsdds increased the production of CoQ(10) as compared to adding vector only.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid comprising a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:1 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (3626, 100), point B has coordinates (3626, 65), point C has coordinates (50, 65), and point D has coordinates (12, 100).

2. The isolated nucleic acid of claim 1, wherein said point B has coordinates (3626, 85).

3. The isolated nucleic acid of claim 1, wherein said point C has coordinates (100, 65).

4. The isolated nucleic acid of claim 1, wherein said point C has coordinates (50, 85).

5. The isolated nucleic acid of claim 1, wherein said point D has coordinates (15, 100).

6. The isolated nucleic acid of claim 1, wherein said nucleic acid sequence encodes a polypeptide.

7. The isolated nucleic acid of claim 6, wherein said polypeptide has DXS activity.

8. The isolated nucleic acid of claim 1, wherein said nucleic acid sequence is as set forth in SEQ ID NO:1.

9. An isolated nucleic acid comprising a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:2 over said length, wherein the

point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1926, 100), point B has coordinates (1926, 65), point C has coordinates (50, 65), and point D has coordinates (12, 100).

10. The isolated nucleic acid of claim 9, wherein said nucleic acid sequence encodes a polypeptide.

11. The isolated nucleic acid of claim 10, wherein said polypeptide has DXS activity.

12. An isolated nucleic acid comprising a nucleic acid sequence, wherein said nucleic acid sequence encodes a polypeptide comprising an amino acid sequence, wherein said amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:3 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (641, 100), point B has coordinates (641, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100).

13. The isolated nucleic acid of claim 12, wherein said polypeptide has DXS activity.

14. An isolated nucleic acid comprising a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:37 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1990, 100), point B has coordinates (1990, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100).

15. The isolated nucleic acid of claim 14, wherein said point B has coordinates (1990, 85).

16. The isolated nucleic acid of claim 14, wherein said point C has coordinates (100,

55).

17. The isolated nucleic acid of claim 14, wherein said point C has coordinates (50, 85).

18. The isolated nucleic acid of claim 14, wherein said point D has coordinates (20, 100).

19. The isolated nucleic acid of claim 14, wherein said nucleic acid sequence encodes a polypeptide.

20. The isolated nucleic acid of claim 19, wherein said polypeptide has DDS activity.

21. The isolated nucleic acid of claim 14, wherein said nucleic acid sequence is as set forth in SEQ ID NO:37.

22. An isolated nucleic acid comprising a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:38 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1002, 100), point B has coordinates (1002, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100).

23. The isolated nucleic acid of claim 22, wherein said nucleic acid sequence encodes a polypeptide.

24. The isolated nucleic acid of claim 23, wherein said polypeptide has DDS activity.

25. An isolated nucleic acid comprising a nucleic acid sequence, wherein said nucleic acid sequence encodes a polypeptide comprising an amino acid sequence, wherein said amino acid sequence has a length and a percent identity to the sequence set forth in SEQ

ID NO:39 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (333, 100), point B has coordinates (333, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100).

26. The isolated nucleic acid of claim 25, wherein said polypeptide has DDS activity.

27. An isolated nucleic acid comprising a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:40 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1833, 100), point B has coordinates (1833, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100).

28. The isolated nucleic acid of claim 27, wherein said point B has coordinates (1833, 85).

29. The isolated nucleic acid of claim 27, wherein said point C has coordinates (100, 65).

30. The isolated nucleic acid of claim 27, wherein said point C has coordinates (50, 85).

31. The isolated nucleic acid of claim 27, wherein said point D has coordinates (20, 100).

32. The isolated nucleic acid of claim 27, wherein said nucleic acid sequence encodes a polypeptide.

33. The isolated nucleic acid of claim 32, wherein said polypeptide has DDS activity.

34. The isolated nucleic acid of claim 27, wherein said nucleic acid sequence is as set forth in SEQ ID NO:40.

35. An isolated nucleic acid comprising a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:41 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1014, 100), point B has coordinates (1014, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100).

36. The isolated nucleic acid of claim 35, wherein said nucleic acid sequence encodes a polypeptide.

37. The isolated nucleic acid of claim 36, wherein said polypeptide has DDS activity.

38. An isolated nucleic acid comprising a nucleic acid sequence, wherein said nucleic acid sequence encodes a polypeptide comprising an amino acid sequence, wherein said amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:42 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (337, 100), point B has coordinates (337, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100).

39. The isolated nucleic acid of claim 38, wherein said polypeptide has DDS activity.

40. An isolated nucleic acid comprising a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:95 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (2017, 100), point B has coordinates (2017, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100).

41. The isolated nucleic acid of claim 40, wherein said point B has coordinates (2017, 85).

42. The isolated nucleic acid of claim 40, wherein said point C has coordinates (100, 65).

43. The isolated nucleic acid of claim 40, wherein said point C has coordinates (50, 85).

44. The isolated nucleic acid of claim 40, wherein said point D has coordinates (20, 100).

45. The isolated nucleic acid of claim 40, wherein said nucleic acid sequence encodes a polypeptide.

46. The isolated nucleic acid of claim 45, wherein said polypeptide has DXR activity.

47. The isolated nucleic acid of claim 40, wherein said nucleic acid sequence is as set forth in SEQ ID NO:95.

48. An isolated nucleic acid comprising a nucleic acid sequence having a length and a percent identity to the sequence set forth in SEQ ID NO:96 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (1161, 100), point B has coordinates (1161, 65), point C has coordinates (50, 65), and point D has coordinates (16, 100).

49. The isolated nucleic acid of claim 48, wherein said nucleic acid sequence encodes a polypeptide.

50. The isolated nucleic acid of claim 49, wherein said polypeptide has DXR activity.

51. An isolated nucleic acid comprising a nucleic acid sequence, wherein said nucleic acid sequence encodes a polypeptide comprising an amino acid sequence, wherein said amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:97 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (386, 100), point B has coordinates (386, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100).

52. The isolated nucleic acid of claim 51, wherein said polypeptide has DXR activity.

53. An isolated nucleic acid comprising a nucleic acid sequence of at least 12 nucleotides, wherein said isolated nucleic acid hybridizes under hybridization conditions to the sense or antisense strand of a nucleic acid molecule, the sequence of said nucleic acid molecule being the sequence set forth in SEQ ID NO: 1, 2, 37, 38, 40, 41, 95, or 96.

54. The isolated nucleic acid of claim 53, wherein said nucleic acid sequence is at least 50 nucleotides.

55. The isolated nucleic acid of claim 53, wherein said nucleic acid sequence encodes a polypeptide.

56. The isolated nucleic acid of claim 53, wherein said polypeptide has DXS, DDS, or DXR activity.

57. A substantially pure polypeptide comprising an amino acid sequence, wherein said amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:3 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (641, 100), point B has coordinates (641, 65), point C has coordinates

(25, 65), and point D has coordinates (5, 100).

58. The substantially pure polypeptide of claim 57, wherein said polypeptide has DXS activity.

59. A substantially pure polypeptide comprising an amino acid sequence, wherein said amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:39 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (333, 100), point B has coordinates (333, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100).

60. The substantially pure polypeptide of claim 59, wherein said polypeptide has DDS activity.

61. A substantially pure polypeptide comprising an amino acid sequence, wherein said amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:42 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (337, 100), point B has coordinates (337, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100).

62. The substantially pure polypeptide of claim 61, wherein said polypeptide has DDS activity.

63. A substantially pure polypeptide comprising an amino acid sequence, wherein said amino acid sequence has a length and a percent identity to the sequence set forth in SEQ ID NO:97 over said length, wherein the point defined by said length and said percent identity is within the area defined by points A, B, C, and D of Figure 26, wherein point A has coordinates (386, 100), point B has coordinates (386, 65), point C has coordinates (25, 65), and point D has coordinates (5, 100).

64. The substantially pure polypeptide of claim 63, wherein said polypeptide has DXR activity.

65. A host cell comprising an isolated nucleic acid of claim 1, 9, 12, 14, 22, 25, 27, 35, 38, 40, 48, 51, or 53.

66. The host cell of claim 65, wherein said host cell is prokaryotic.

67. The host cell of claim 65, wherein said host cell is selected from the group consisting of *Rhodobacter*, *Sphingomonas*, and *Escherichia* cells.

68. The host cell of claim 65, wherein said host cell comprises an exogenous nucleic acid that encodes a polypeptide having DDS, DXS, ODS, SDS, DXR, 4-diphosphocytidyl-2C-methyl-D-erythritol synthase, 4-diphosphocytidyl-2C-methyl-D-erythritol kinase, or chorismate lyase activity.

69. The host cell of claim 65, wherein said host cell comprises an exogenous nucleic acid comprising an UbiC sequence or LytB sequence.

70. The host cell of claim 65, wherein said host cell comprises an exogenous nucleic acid comprising an UbiC sequence and LytB sequence.

71. The host cell of claim 65, wherein said host cell comprises non-functional crtE sequence, ppsR sequence, or ccoN sequence.

72. The host cell of claim 65, wherein said host cell comprises non-functional crtE sequence, ppsR sequence, and ccoN sequence.

73. A host cell comprising an exogenous nucleic acid and a non-functional crtE sequence, ppsR sequence, or ccoN sequence, wherein said exogenous nucleic acid is

within a crtE, ppsR, or ccoN locus of said host cell.

74. A host cell comprising a genomic deletion, wherein said deletion comprises at least a portion of a crtE sequence, ppsR sequence, or ccoN sequence, and wherein said host cell comprises a non-functional crtE sequence, ppsR sequence, or ccoN sequence.

75. A method for increasing production of CoQ(10) in a cell having endogenous DDS activity, said method comprising inserting a nucleic acid molecule comprising a nucleic acid sequence that encodes a polypeptide having DDS activity into said cell such that production of CoQ(10) is increased.

76. The method of claim 75, wherein said nucleic acid molecule comprises an isolated nucleic acid of claim 14, 22, 25, 27, 35, 38, or 53.

77. The method of claim 75, wherein the production of CoQ(10) is increased at least about 5 percent as compared to a control cell lacking said inserted nucleic acid molecule.

78. The method of claim 75, wherein said cell is selected from the group consisting of *Rhodobacter* and *Sphingomonas* cells.

79. The method of claim 75, wherein said cell is a membraneous bacterium.

80. The method of claim 75, wherein said cell is a highly membraneous bacterium.

81. The method of claim 75, wherein said method further comprises inserting a second nucleic acid molecule comprising a nucleotide sequence that encodes a polypeptide having DXS activity into said cell.

82. The method of claim 81, wherein said second nucleic acid molecule comprises an isolated nucleic acid of claim 1, 9, or 12.

83. A method for increasing production of CoQ(10) in a cell having endogenous DDS activity, said method comprising inserting a nucleic acid molecule comprising a nucleic acid sequence that encodes a polypeptide having DXS activity into said cell such that production of CoQ(10) is increased.

84. The method of claim 83, wherein the production of CoQ(10) is increased at least about 5 percent as compared to a control cell lacking said inserted nucleic acid molecule.

85. The method of claim 83, wherein said cell is selected from the group consisting of *Rhodobacter* and *Sphingomonas* cells.

86. The method of claim 83, wherein said nucleic acid molecule comprises an isolated nucleic acid of claim 1, 9, or 12.

87. The method of claim 83, wherein said cell is a membraneous bacterium.

88. The method of claim 83, wherein said cell is a highly membraneous bacterium.

89. The method of claim 83, wherein said method further comprises inserting a second nucleic acid molecule comprising a nucleotide sequence that encodes a polypeptide having DDS activity into said cell.

90. The method of claim 89, wherein said second nucleic acid molecule comprises an isolated nucleic acid of claim 14, 22, 25, 27, 35, 38, or 53.

91. A method for increasing production of CoQ(10) in a membraneous bacterium, said method comprising inserting a nucleic acid molecule comprising a nucleic acid sequence that encodes a polypeptide having DDS activity into said bacterium such that production of CoQ(10) is increased.

92. A method for increasing production of CoQ(10) in a highly membraneous

bacterium, said method comprising inserting a nucleic acid molecule comprising a nucleic acid sequence that encodes a polypeptide having DDS activity into said highly membraneous bacterium such that production of CoQ(10) is increased.

93. A method for making an isoprenoid, said method comprising culturing a cell under conditions wherein said cell produces said isoprenoid, said cell comprising at least one exogenous nucleic acid that encodes at least one polypeptide, wherein said cell produces more of said isoprenoid than a comparable cell lacking said at least one exogenous nucleic acid.

94. The method of claim 93, wherein said cell is selected from the group consisting of *Rhodobacter* and *Sphingomonas* cells.

95. The method of claim 93, wherein said isoprenoid is CoQ(10).

96. The method of claim 93, wherein said at least one polypeptide has DDS, DXS, ODS, SDS, DXR, 4-diphosphocytidyl-2C-methyl-D-erythritol synthase, 4-diphosphocytidyl-2C-methyl-D-erythritol kinase, or chorismate lyase activity.

97. The method of claim 93, wherein said at least one polypeptide is a UbiC polypeptide or a LytB polypeptide.

98. The method of claim 93, wherein said cell comprises a non-functional crtE sequence, ppsR sequence, or ccoN sequence.

99. The method of claim 93, wherein said cell comprises a non-functional crtE sequence, ppsR sequence, and ccoN sequence.

100. The method of claim 93, wherein said cell comprising a genomic deletion, wherein said deletion comprises at least a portion of a crtE sequence, ppsR sequence, or ccoN sequence, and wherein said cell comprises a non-functional crtE sequence, ppsR

sequence, or ccoN sequence.

101. A method for making an isoprenoid, said method comprising culturing a genetically modified cell under conditions wherein said cell produces said isoprenoid.

102. The method of claim 101, wherein said isoprenoid is CoQ(10).

103. The method of claim 101, wherein said cell comprises an exogenous nucleic acid.

104. The method of claim 101, wherein said cell comprises a genomic deletion.

Figure 1

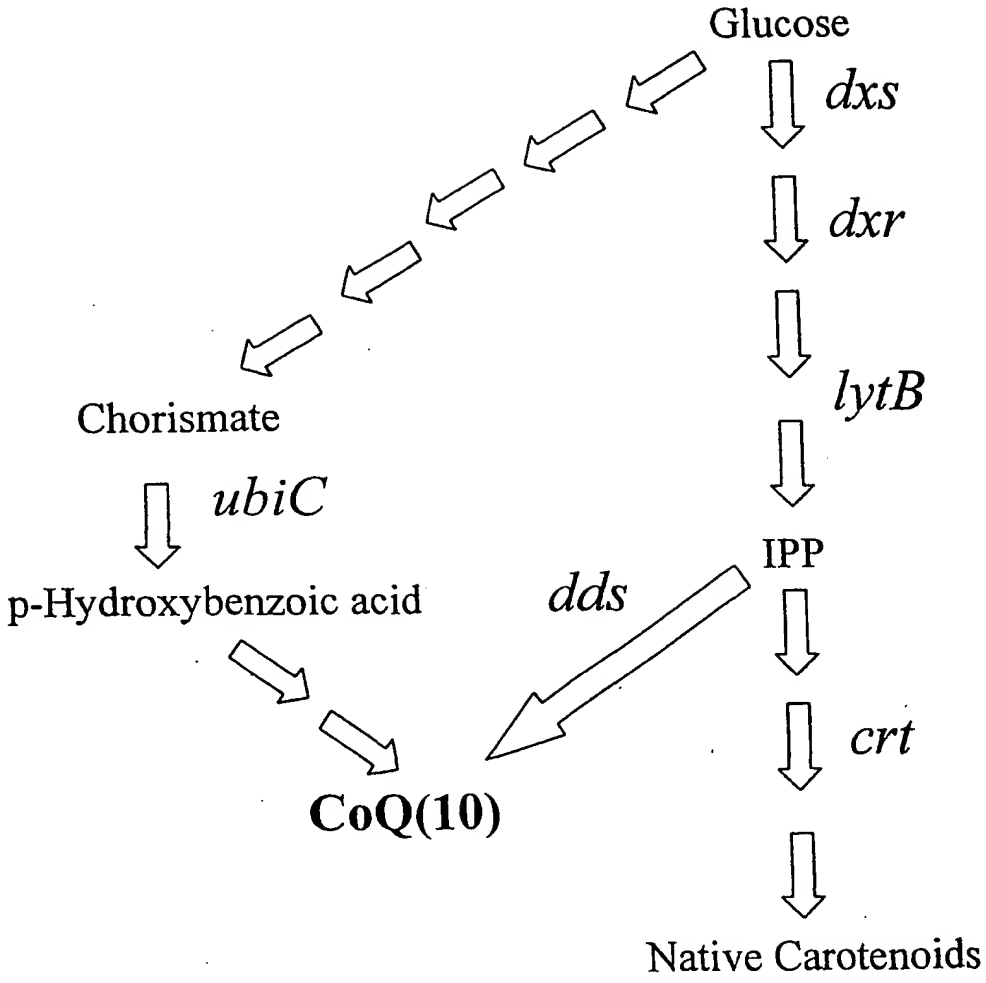
Glucose
dxs
dxr
lytB
IPP
Chorismate
ubiC
p-Hydroxybenzoic acid
dds
crt
CoQ(10)
Native Carotenoids

Figure 2 (page 1 of 2)

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   1  ctgcggccag accacgcata tcgacgacga ttcgatcacg aaaaacgtac
  51  ggtccgcagc ccagcacgcc ggtttttcgc cggtccggcc ggtgatcgag
 101  gtgcgcggca agtgcggcaa gtgtgactga cctgtccaac agaccgttcg
 151  acttgagact aacgttgcgc taacaaagcc catggctgac ctacccaaga
 201  cgccgctgct cgacacggtc gacacgccgc aggacctccg gaagctcgcc
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2051  gtgctgaagg cgctccgcta caacgaggcc gagctggccg acggggtgcg
2101  ggcgtaaacg acgccagatc ctccccggaa cggggagggg aaccgccgcc
2151  gaaggcggtg gtggaggggc cgctgcggca cgcancggtt tcccaggctg
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Figure 2 (page 2 of 2)

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2251  atggtccccc tccccgttcc ggggaggatc tgggtcctgc cccaccttga
2301  atctccaaca tgcacatgcc atgtacatgc acatggctac gcagcttccc
2351  cagactcgct ccagccgcgt tgtcgtgctg gtatcgcccg aggaaaaacg
2401  gcgcatttcc gccaatgcgg aagcggcgga catgacggtc agcgacttca
2451  tgcgcaccgc cgccgaacgc tataccgagc cgaccgacgc cgagatggcg
2501  ctgatgcgcg acctgctcgc ccagctcgaa caggccaatg cccgcacgga
2551  cgcggccttt gcccagctcg aagctgcgcg cgccgccgcc accgcgttcg
2601  acgaggaggc gtatcgcgcc gaggtccgcg aacagctgct gaccgatacc
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2901  cgaaagtcca caaaccggca gctagcgccc gcttccccga gcgcgtacat
2951  cgcggtacgt gctgaaaatg accatccttc ccctcaccgc ccgccccgc
3001  gcgctcgcgc actggctgtt cgtcgtcgcc gcgatgatcg tcgcgatggt
3051  cgtggtcggg ggcattaccc ggctcaccga atcgggcctg tcgatcaccg
3101  aatggaagcc aatctccggc atcgtgcccc cgctcaacga cgcgcagtgg
3151  caggccgagt tcgaccacta caagcagatc ggccagtatg agcagctcaa
3201  ccagggcatg acgctcggcg ggttcaagag catcttcttc tgggaatata
3251  tccaccgcct gctcggccgg ctgatcggca tggtgttcgc gctgccgctg
3301  ctgtggttcg ccgtccgcaa gcagatcccg cagggctatg gctggcggct
3351  ggtcgcgctg ctcgcgctag gcgggctgca gggcgcgttc ggctggtgga
3401  tggtgaagtc ggggctcaac cacacccgca cctcggttag ccatttctgg
3451  ctggcgaccc acctgatgac cgcactgttc acgctgggcg gcatcgtctg
3501  gacgatgctc gacctgcgcg cgcttgccgc caaccatgcc gagcgccctg
3551  cccgactgac cgggctcggc gcgggcgtgc tggtactgct ggcggtccag
3601  ctcttctacg gggcgctggt agcagg  (SEQ ID NO:1)
```

Figure 3

```
   1  atggctgacc tacccaagac gccgctgctc gacacggtcg acacgccgca
  51  ggacctccgg aagctcgccc ccgcccagct gcgccagctg gccgacgagc
 101  ttcgtgccga aaccatcagt gcggtgggct ccaccggcgg gcatctaggc
 151  tccggcctgg gcgtcgtcga actgacggtg gcgatccact atgtattcaa
 201  cacccccgac gaccggctga tctgggacgt cgggcaccaa tgctatccgc
 251  acaagatcct caccggtcgg cgcgatcgga tccgcacgat tcgtcagggt
 301  ggaggcctct ccggcttcac caagcgcagc gagagcgagt atgatccgtt
 351  cggtgccgcg cactcgtcga cctcgatctc ggccgcactc ggctttgcga
 401  tcgccaacaa gctcaacgag gcgccgggca aggcgatcgc ggtgatcggc
 451  gacggcgcga tgagcgcggg catggcctat gaggcgatga acaacgccga
 501  ggccgccggc aaccggctgg tggtgatcct caacgacaac gacatgtcga
 551  tcgccccgcc ggtgggcggg ctttcggcct atcttgcgcg cctcatttcc
 601  tcgtccgaat atctcggcct gcgcgagctc gccaagcgct tcacccgcaa
 651  gctttcgcgc cgcctcaccg cggcagccgg caaggcggag gaattcgccc
 701  gcggcatggc gaccggcggc acgctgttcg aggaacttgg cttctattat
 751  gtcggcccga tcgacggcca caatctcgag catctgatcc cggtgctgga
 801  gaatgtccgc gacagcgagc agggcccgat cctgatccat gtcgtgacca
 851  agaagggcaa gggctatgcc ccggccgaag cggcggcgga caagtatcac
 901  ggcgtccaga agttcgacgt gatcaccggg gcacaggcca aggcaccccc
 951  gggcccgccc gcctatacca aggtgttcgc cgatgcgctg ctcgccgaag
1001  cggagcgtga tgcgtcggtc tgcgcgatca ccgcggcgat gccctcgggc
1051  accgggctcg acaagttcca ggcgacgttc cccgatcgca ccttcgacgt
1101  gggcattgcc gaacagcacg cggtcacctt cgcagcgggc cttgccgcgc
1151  aggggatgcg gccgttctgc gcgatctact cgaccttcct gcagcgcgcc
1201  tacgaccagg tcgtccacga cgtcgcgatc cagaacctgc cggtccgctt
1251  cgcgatcgac cgcgcgggcc tggtcggtgc cgacggcgcg acccatgccg
1301  gcagcttcga cgtgacctat ctcgccagcc tgcccaattt cgtggtgatg
1351  gcggccgcgg acgaggtcga gctcgtccac atgacccaca cggcggcgat
1401  gcacgacagc ggcccgatcg cgctgcgcta tccacgcggc aacggcgtcg
1451  gactggcgct gcccaaggtt ccggagcggc tggaaatcgg caagggtcgc
1501  gtggtccgag agggcaagaa ggtagcgatc ctgtcgctcg gcacgcgcct
1551  tgcggaagca ctaaaggccg ccgacacgct cgaggccaag ggcctctcga
1601  ccaccgtcgc cgacctgcgc ttcgccaaac cgctcgacga ggatctgatc
1651  cgccgcctgc tcaccaccca cgaagtggcg gtgacgatcg aggaaggcgc
1701  gatcggcggc cccggtgcgc atgtgctgac gctcgccagc gataccggcc
1751  tgatcgacgc cggcctcaag ctgcgcacca tgcgcctgcc ggacatattc
1801  caggaccagg acaagcccga gaagcagtat gacgaagcgg ggctgaacgc
1851  cgccaacatc gtcgacacgg tgctgaaggc gctccgctac aacgaggccg
1901  agctggccga cggggtgcgg gcgtaa  (SEQ ID NO:2)
```

Figure 4

```
  1  madlpktpll dtvdtpqdlr klapaqlrql adelraetis avgstgghlg
 51  sglgvveltv aihyvfntpd drliwdvghq cyphkiltgr rdrirtirqg
101  gglsgftkrs eseydpfgaa hsstsisaal gfaianklne apgkaiavig
151  dgamsagmay eamnnaeaag nrlvvilndn dmsiappvgg lsaylarlis
201  sseylglrel akrftrklsr rltaaagkae efargmatgg tlfeelgfyy
251  vgpidghnle hlipvlenvr dseqgpilih vvtkkgkgya paeaaadkyh
301  gvqkfdvitg aqakappgpp aytkvfadal laeaerdasv caitaampsg
351  tgldkfqatf pdrtfdvgia eqhavtfaag laaqgmrpfc aiystflqra
401  ydqvvhdvai qnlpvrfaid raglvgadga thagsfdvty laslpnfvvm
451  aaadevelvh mthtaamhds gpialryprg ngvglalpkv perleigkgr
501  vvregkkvai lslgtrlaea lkaadtleak glsttvadlr fakpldedli
551  rrllttheva vtieegaigg pgahvltlas dtglidaglk lrtmrlpdif
601  qdqdkpekqy deaglnaani vdtvlkalry neaeladgvr a (SEQ ID
NO:3)
```

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```
STdxsdna        182 atg-----------------------------------
CRdxsdna          1 atgctgcgtggtgctgtttctcacggccctgcggtcgccg
CJdxsdna          1 -----------------------------------------
PAdxsdna          1 atg-----------------------------------
LEdxsdna          1 atg-----------------------------------
MTdxsdna          1 -----------------------------------------
RSdxs1dna         1 atg-----------------------------------
RSdxs2dna         1 atg-----------------------------------
SPCCdxsdna        1 -----------------------------------------
ECdxsdna          1 atg-----------------------------------
NMdxsdna          1 -----------------------------------------
HIdxsdna          1 atg-----------------------------------
SSdxsdna          1 ---------------------------------------
HPdxsdna          1 -----------------------------------------

STdxsdna        185 ----------gct-------------------------
CRdxsdna         41 accgggctgccgct-------------------------
CJdxsdna          1 ----------at--------------------------
PAdxsdna          4 ----------cccaagacgctccatgagattccccgc--
LEdxsdna          4 ----------gctttgtgtgcttatgcatttcctgggat
MTdxsdna          1 -----------------------------------------
RSdxs1dna         4 ----------acc-------------------------
RSdxs2dna         4 ----------acc-------------------------
SPCCdxsdna        1 -----------------------------------------
ECdxsdna          4 ----------agtttt----------------------
NMdxsdna          1 -----------------------------------------
HIdxsdna          4 ----------act-------------------------
SSdxsdna          1 -----------------------------------------
HPdxsdna          1 ----------gt--------------------------

STdxsdna        188 -----------------------------------------
CRdxsdna         55 -----------------------------------------
CJdxsdna          3 -----------------------------------------
PAdxsdna         31 -----------------------------------------
LEdxsdna         33 tttgaacaggactggtgtggtttcagattcttctaaggca
MTdxsdna          1 -----------------------------------------
RSdxs1dna         7 -----------------------------------------
RSdxs2dna         7 -----------------------------------------
SPCCdxsdna        1 -----------------------------------------
ECdxsdna         10 -----------------------------------------
NMdxsdna          1 -----------------------------------------
HIdxsdna          7 -----------------------------------------
SSdxsdna          1 -----------------------------------------
HPdxsdna          3 -----------------------------------------
```

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```
STdxsdna      188 ---------------------------------gacc---
CRdxsdna       55 ---------------------------------ggcc---
CJdxsdna        3 ---------------------------------ga-g---
PAdxsdna       31 ---------------------------------gagc---
LEdxsdna       73 acccctttgttctctggatggattcatggaacagatc---
MTdxsdna        1 ------------------------------------------
RSdxs1dna       7 ---------------------------------gaca---
RSdxs2dna       7 ---------------------------------aatc---
SPCCdxsdna      1 ------------------------------------------
ECdxsdna       10 ---------------------------------gata---
NMdxsdna        1 ---------------------------------atg---
HIdxsdna        7 ---------------------------------aacaata
SSdxsdna        1 ------------------------------------------
HPdxsdna        3 ---------------------------------gatt---

STdxsdna      192 t---------ac----------cc----------------
CRdxsdna       59 c---------cg----------cccgctgcgctgctcccg
CJdxsdna        6 t---------aa----------aa----------------
PAdxsdna       35 g---------cc----------cc----------------
LEdxsdna      110 t---------gcagtttttgttcc----------------
MTdxsdna        1 ------------------------------------------
RSdxs1dna      11 g---------ac----------cc----------------
RSdxs2dna      11 ccaccccgcgac----------cc----------------
SPCCdxsdna      1 ------------------------------------------
ECdxsdna       14 t---------tg----------cc----------------
NMdxsdna        4 a---------ac----------cc----------------
HIdxsdna       14 t---------ga----------ac----------------
SSdxsdna        1 ------------------------------------------
HPdxsdna        7 t---------tg----------ca----------------

STdxsdna      197 ------------------------------------------
CRdxsdna       80 tcgcccgtggtgtgcgcagcgcagcgcccacgcgtcagcg
CJdxsdna       11 ------------------------------------------
PAdxsdna       40 ------------------------------------------
LEdxsdna      125 ------------------------------------------
MTdxsdna        1 ------------------------------------------
RSdxs1dna      16 ------------------------------------------
RSdxs2dna      25 ------------------------------------------
SPCCdxsdna      1 ------------------------------------------
ECdxsdna       19 ------------------------------------------
NMdxsdna        9 ------------------------------------------
HIdxsdna       19 ------------------------------------------
SSdxsdna        1 ------------------------------------------
HPdxsdna       12 ------------------------------------------
```

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```
STdxsdna      197 ----------------------------------------
CRdxsdna      120 tcgcgcggaggcttcggtcaatgccccgcgggcgggcccg
CJdxsdna       11 ----------------------------------------
PAdxsdna       40 ----------------------------------------
LEdxsdna      125 ----------------------------------------
MTdxsdna        1 ----------------------------------------
RSdxs1dna      16 ----------------------------------------
RSdxs2dna      25 ----------------------------------------
SPCCdxsdna      1 ----------------------------------------
ECdxsdna       19 ----------------------------------------
NMdxsdna        9 ----------------------------------------
HIdxsdna       19 ----------------------------------------
SSdxsdna        1 ----------------------------------------
HPdxsdna       12 ----------------------------------------

STdxsdna      197 ----------------------------------------
CRdxsdna      160 gccggtagctactcgggcgagtgggataagctttcagtgg
CJdxsdna       11 ----------------------------------------
PAdxsdna       40 ----------------------------------------
LEdxsdna      125 ----------------------------------------
MTdxsdna        1 ----------------------------------------
RSdxs1dna      16 ----------------------------------------
RSdxs2dna      25 ----------------------------------------
SPCCdxsdna      1 ----------------------------------------
ECdxsdna       19 ----------------------------------------
NMdxsdna        9 ----------------------------------------
HIdxsdna       19 ----------------------------------------
SSdxsdna        1 ----------------------------------------
HPdxsdna       12 ----------------------------------------

STdxsdna      197 --------------------------------aag--acg
CRdxsdna      200 aggagattgatgagtggcgcgatgtgggcccgaag--acg
CJdxsdna       11 --------------------------------aat--ttg
PAdxsdna       40 --------------------------------gcc--acg
LEdxsdna      125 --------------------------------aac--aca
MTdxsdna        1 ----------------------------------------
RSdxs1dna      16 --------------------------------tgc--acg
RSdxs2dna      25 --------------------------------gaa--acc
SPCCdxsdna      1 ------------------------------------atg
ECdxsdna       19 --------------------------------aaa--tac
NMdxsdna        9 --------------------------------aag----c
HIdxsdna       19 --------------------------------aat--tat
SSdxsdna        1 ------------------------------------gtg
HPdxsdna       12 --------------------------------aaataaaa
```

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```
STdxsdna      203 ccgctgctc----------gacacggtcga--------ca
CRdxsdna      238 cccctgctg----------gacactgtcaa--------tt
CJdxsdna       17 cccatactc----------aa------------------
PAdxsdna       46 cccctgctc----------gaccgcgcctc--------tt
LEdxsdna      131 agcttactcatgaggtcaagaaaaggtcacgtgtggttca
MTdxsdna        1 ---atgctg----------caacagatccg--------cg
RSdxs1dna      22 ccgacgctc----------gac-cgggtga--------cg
RSdxs2dna      31 ccgcttttg----------gatcgcgtctg--------ct
SPCCdxsdna      4 catctcagc----------gaaa---ttac--------cc
ECdxsdna       25 ccgaccctg----------gcactggtcga--------ct
NMdxsdna       13 cccctactc----------gacctgattga--------ca
HIdxsdna       25 cctctttta----------tctttaattaa--------tt
SSdxsdna        4 acgattctg----------gagaacatccg--------gc
HPdxsdna       20 cttttgatt----------taaaccctaac--------ga

STdxsdna      225 cgcc-gcaggacc----tccgga---------------ag
CRdxsdna      260 accc-ggtgcacc----tgaaga---------------ac
CJdxsdna       28 ------gaagagt----tagaaa---------------ag
PAdxsdna       68 cgcc-ggccgaac----tgcgcc---------------gg
LEdxsdna      171 ggct-tccttatcagaatctggagaatactacacacagag
MTdxsdna       20 ggcc-cgctgatc----tgcagc---------------ac
RSdxs1dna      43 ctcccggtggaca----taaagg---------------gc
RSdxs2dna      53 gccc-ggccgaca----tgaagg---------------cg
SPCCdxsdna     23 atcc-caaccagc----tccacg---------------gg
ECdxsdna       47 ccac-ccaggagt----tacgac---------------tg
NMdxsdna       35 gccc-gcaagatt----tgcgcc---------------gt
HIdxsdna       47 ctcc-agaagatt----tgcgtc---------------tt
SSdxsdna       26 aacc-acgcgacc----tgaagg---------------cg
HPdxsdna       42 tatt-gcagg--------cttgg---------------ag

STdxsdna      245 ctcgcccccgcccagctgcgccag----------------
CRdxsdna      280 ttcaacaatgagcagctgaagcag----------------
CJdxsdna       43 ctaagtttaaaagaattagaaaat----------------
PAdxsdna       88 ctgggcgaggcggacctggaaacc----------------
LEdxsdna      210 accgccaacgcctattttggacactgtgaactatcccatt
MTdxsdna       40 ctttcccaggcgcagcttcgggag----------------
RSdxs1dna      64 ctcacggaccgtgagttgcgctcg----------------
RSdxs2dna      73 ctgagtgacgccgaactggagcgg----------------
SPCCdxsdna     43 ttgtcggttgctcagcttgagcaa----------------
ECdxsdna       67 ttgccgaaagagagtttaccgaaa----------------
NMdxsdna       55 ctggacaaaaaacagctgccgcgc----------------
HIdxsdna       67 ttaaataaagatcagctaccacaa----------------
SSdxsdna       46 ctgcccgaggagcagctgcacgaa----------------
HPdxsdna       58 tt------------ggtgtgtcaa----------------
```

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```
STdxsdna      269 ------------------------------------ctgg
CRdxsdna      304 ------------------------------------ctct
CJdxsdna       67 ------------------------------------ttag
PAdxsdna      112 ------------------------------------ctgg
LEdxsdna      250 catatgaaaaatctgtctctgaaggaacttaaacaactag
MTdxsdna       64 ------------------------------------ctgg
RSdxs1dna      88 ------------------------------------ctgg
RSdxs2dna      97 ------------------------------------ctgg
SPCCdxsdna     67 ------------------------------------attg
ECdxsdna       91 ------------------------------------ctct
NMdxsdna       79 ------------------------------------cttg
HIdxsdna       91 ------------------------------------ctct
SSdxsdna       70 ------------------------------------ctgt
HPdxsdna       70 ------------------------------------acg-

STdxsdna      273 ccgacgagcttcgtgccgaaacca-tcagtg--cggtggg
CRdxsdna      308 gcaaggagctgcgcagtgacatcg-tgcaca--ccgtctc
CJdxsdna       71 cagcatctatgcgtgaaaaaatca-tacaag--ttgtgag
PAdxsdna      116 ccgacgagct--gcgccagtacct-gctgtataccgtcgg
LEdxsdna      290 cagatgaactaaggtcagatacaa-ttttca--atgtatc
MTdxsdna       68 ccgccgagatccgtgagttcctga-tccaca--aggttgc
RSdxs1dna      92 ccgacgagctgcgggccgaaacga-tctcgg--ccgtgtc
RSdxs2dna     101 ccgacgaagtgcgttccgaggtga-tttcgg--tcgttgc
SPCCdxsdna     71 gccaccagattcgtgagaagcacc-tgcaga--cggttgc
ECdxsdna       95 gcgacgaactgcgccgctatttac-tcgaca--gcgtgag
NMdxsdna       83 ccggcgagttgcgcacctttctgc-tggaat--ctgtcgg
HIdxsdna       95 gtcaagaattacgtgcttatcttt-tagaat--ctgttag
SSdxsdna       74 ccgaggaga-tcaggcagttcctggtgcacg--cggtcac
HPdxsdna       73 -ctacg-gaatcgt------attt-tagaag--tggtgag

STdxsdna      310 ctccaccggcgggcatctaggctccggcctgggcgtcgtc
CRdxsdna      345 tcgcaccggtggacaccttagcagcagcctgggcgtggtg
CJdxsdna      108 taaaaatggtgggcatttaagttcaaatttgggtgctgta
PAdxsdna      153 ccagaccggcggtcatttcggcgccggcctcggcgtggtc
LEdxsdna      327 aaagactgggggtcaccttggctcaagtcttggtgttgtt
MTdxsdna      105 cgccacgggggggcatctggggccgaacctgggagtggtg
RSdxs1dna     129 ggtgacgggcgggcatctgggcgcaggcctcggcgtggtg
RSdxs2dna     138 cgagacgggaggacatctggggtcctcgctgggggtggtt
SPCCdxsdna    108 agcgaccggtgggcacctcgggccgggcttgggcgtggtg
ECdxsdna      132 ccgttccagcgggcacttcgcctccgggctgggcacggtc
NMdxsdna      120 gcagaccggcgggcatttcgccagcaatttgggcgcggtc
HIdxsdna      132 tcaaactagcggacatttagcgtcaggtttaggcactgta
SSdxsdna      111 cagaaccggcggtcatctgggacccaacctgggggtggtg
HPdxsdna      102 cgctaatgggggggcatttaagctcttctttaggggctgtg
```

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```
STdxsdna        350 gaactgacggtggcgatccactatgtattcaacaccccg
CRdxsdna        385 gagctgacggtggctatgcactatgtattcaacaccccgg
CJdxsdna        148 gaacttagtatagcaatgcatttggtttttgatgcaaaaa
PAdxsdna        193 gagctgaccattgccctgcactacgtcttcgacactccgg
LEdxsdna        367 gagctgactgttgctcttcattatgtcttcaatgcaccgc
MTdxsdna        145 gaactcaccttggcgctgcaccgggtattcgactcgccgc
RSdxs1dna       169 gagttgacggttgcgctgcatgcgatcttcgatgcgcccc
RSdxs2dna       178 gagctgactgtcgcgctgcatgcggtcttcaacacgccca
SPCCdxsdna      148 gaattgaccctagcgctttaccaaacgctcgatctcgatc
ECdxsdna        172 gaactgaccgtggcgctgcactatgtctacaacaccccgt
NMdxsdna        160 gagctgacggttgcgctgcactacgtttacaacacgcccg
HIdxsdna        172 gagctaaccgttgcgctgcattatgtatataagacgccat
SSdxsdna        151 gagctgaccatcgccctgcaccgggtcttcgagtcgcccg
HPdxsdna        142 gagctgattgtgggcatgcatgccttatttgattgccaaa

STdxsdna        390 acgaccggctgatctgggacgtcgggcaccaatgctatcc
CRdxsdna        425 aggacaagattatttgggacgtgggccaccaggcgtatgg
CJdxsdna        188 aagatccttttatttttgatgtgtcgcatcagtcttatac
PAdxsdna        233 acgaccgcctggtctgggacgtcggccaccaggcctatcc
LEdxsdna        407 aagataggattctctgggatgttggtcatcagtcttatcc
MTdxsdna        185 acgatccgatcatcttcgacaccggtcaccaggcctacgt
RSdxs1dna       209 gcgacaagatcatctgggacgtgggccaccagtgctaccc
RSdxs2dna       218 ccgacaagctcgtctgggacgtgggccaccagtgctaccc
SPCCdxsdna      188 gcgacaaagtggtttgggacgttggccaccaagcctatcc
ECdxsdna        212 ttgaccaattgatttgggatgtggggcatcaggcttatcc
NMdxsdna        200 aagacaagctggtgtgggatgtcggacaccaaagctatcc
HIdxsdna        212 ttgatcagttaatttgggatgtgggacatcaagcttatcc
SSdxsdna        191 tcgaccgcatcctgtgggacaccggccaccagagctacgt
HPdxsdna        182 aaaacccttttcatttttgacacttcgcaccaagcttacgc

STdxsdna        430 gcacaagatcctcaccggtcggcgcgatcgga---tccgc
CRdxsdna        465 ccacaagatcctgactggccgtcgcaagggta---tggcc
CJdxsdna        228 acacaagcttttaagcggaaaagaagaaatat---ttgat
PAdxsdna        273 gcacaagatcctcaccgagcgccgcgagctga---tgggc
LEdxsdna        447 tcacaaaatcttgactggtagaagggacaaga---tgtcg
MTdxsdna        225 ccacaagatgttgaccggacgcagccaggact---tcgca
RSdxs1dna       249 ccacaagatcctgaccgggcggcgcgaccgca---tccgc
RSdxs2dna       258 ccacaagatcctcaccggccggcgcgagcaga---tgcgc
SPCCdxsdna      228 ccacaagctgctgacag---ggcgctatcacaacttccat
ECdxsdna        252 gcataaaattttgaccggacgccgcgacaaaa---tcggc
NMdxsdna        240 gcacaaaattcttaccggacgtaaaaaccaga---tgcac
HIdxsdna        252 acataaaatcctaacgggtcgccgagagcaaa---tgtcc
SSdxsdna        231 acacaagctgctgacgggacgtcagga---ct---tctcc
HPdxsdna        222 ccacaagcttttaaccgggcgctttgaaagct---ttagc
```

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```
STdxsdna        467 acgattcgtcagggtggaggcctctccggcttcaccaag-
CRdxsdna        502 acgattcgccagaccaacggcctttcgggcttcacgaag-
CJdxsdna        265 actttaagacaaatcaatggtttaagtggttatacaaaa-
PAdxsdna        310 accctgcgccagaagaacggcctggcggccttcccgcgc-
LEdxsdna        484 acattaaggcagacagatggtcttgcaggatttactaag-
MTdxsdna        262 accctgcgtaagaagggcgggttgtcggggtatccgtct-
RSdxs1dna       286 accctgcggcagggcgggggtctctcgggcttcaccaag-
RSdxs2dna       295 accctgcgccagaagggcggcctctcgggcttcaccaag-
SPCCdxsdna      265 accttgcggcaaaaggatggcattgcgggctacccgaag-
ECdxsdna        289 accatccgtcagaaaggcggtctgcacccgttcccgtgg-
NMdxsdna        277 accatgcgccaatatggcggtttggcgggttttccgaaa-
HIdxsdna        289 acaattcgccaaaaagacggtat-tcatccttttccttgg
SSdxsdna        265 aagctgcgcggcaagggcggcctgtccggctacccctcg-
HPdxsdna        259 actttaaggcaattcaagggtttgagcggctttactaaa-

STdxsdna        506 cgcagcgagagcgagtatgatccgttcggtgccgcgc-ac
CRdxsdna        541 cgcgacgagagcgagtacgacccttcggcgctggcc-ac
CJdxsdna        304 cctagcgagggagattat------tttgtagcagggc-at
PAdxsdna        349 cgcgcagagagcgagtacgacaccttcggcgtcggcc-ac
LEdxsdna        523 cgatcggagagtgaatatgattgctttggcaccggcc-ac
MTdxsdna        301 cgtgccgagagcgagcacga-ctgggtggagtcgagccac
RSdxs1dna       325 cgctccgagagcccctatgactgtttcggcgcgggcc-at
RSdxs2dna       334 cgctcggaatccgcctacgacccgttcggcgcggctc-at
SPCCdxsdna      304 cgcacggaaaaccgcttcgatcatttcggtgccggtc-ac
ECdxsdna        328 cgcggcgaaagcgaatatgacgtattaagcgtcgggc-at
NMdxsdna        316 cgttgcgagtccgagtacgacgcgttcggcgtggggc-at
HIdxsdna        328 cgtgaagaaagtgaatttgatgtattaagtgttggtc-ac
SSdxsdna        304 cgcgaggagtccgagcacgacgtcatcgagaacagcc-ac
HPdxsdna        298 cccagcgagagcgcatacgattatttcatcgccgggc-at

STdxsdna        545 tcgtcgacctcgatctcggccgcact--cggctttgcgat
CRdxsdna        580 agctccacctcgatttcggcggctct--gggtatggcggt
CJdxsdna        337 tctagtacctctatttctttggcagt--aggtgcttgtaa
PAdxsdna        388 tccagcacctccatcagcgccgccct--gggcatggccat
LEdxsdna        562 agttccaccaccatctcagcaggcct--agggatggctgt
MTdxsdna        340 gccagcgcggcgctgtcgtacgcgga--cgggttggccaa
RSdxs1dna       364 tcctcgacctcgatctcggccgcggt--gggctttgccgc
RSdxs2dna       373 tcctcgacctcgatctcggccgcgct--cggctttgccat
SPCCdxsdna      343 gcttccaccagtatttctgctggcct--cggtatggctct
ECdxsdna        367 tcatcaacctccatcagtgccggaat--tggtattgcggt
NMdxsdna        355 tcctccacctccatcggcgcggcgtt--gggcatggcggc
HIdxsdna        367 tcctctacgtctattagtgcgggatt--aggcattgccgt
SSdxsdna        343 gcctccac--cgccctcggctgggccgacggactcgccaa
HPdxsdna        337 agttccacttcggtgt------ctat--aggcgttggggt
```

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```
STdxsdna        583 cgc-c-------aacaagctc--------aacgag-gc--
CRdxsdna        618 ggg-c-------cgcgacgtt--------aagggc-aa--
CJdxsdna        375 ggc-t-------attgcttta--------aagggt-ga--
PAdxsdna        426 cgc-c-------gcccgcctg--------caaggc-aa--
LEdxsdna        600 tgg-t-------agagatcta--------aaagga-ag--
MTdxsdna        378 ggc-g-------ttcgagttg--------accg-g-ac--
RSdxs1dna       402 ggc-a-------cgcgagatg--------ggcggc-ga--
RSdxs2dna       411 ggg-t-------cgcgagctg--------ggccag-cc--
SPCCdxsdna      381 agcac-------gggatgccc--------agggcg-aa--
ECdxsdna        405 tgc-tgccgaaaaagaaggca--------aa--aa-tc--
NMdxsdna        393 ggc-g-------gacaaacag--------ttgggcagc--
HIdxsdna        405 tgc-c-------------gcag--------aacgag-aaaa
SSdxsdna        381 ggc-c-------cgccgggtg--------cagggg-ga--
HPdxsdna        369 ggc-t-------a--aagctttttgtttgaaacaa-gc--

STdxsdna        604 -gccgg--gcaaggc----gatcgcggtgatcggcgacgg
CRdxsdna        639 -gaaga--acagtgt----gatcgctgtcatcggcgacgg
CJdxsdna        396 -aaagc--gtattcc----tgttgctttgattggagatgg
PAdxsdna        447 -ggagc--gtaagtc----ggtggccgtgatcggcgacgg
LEdxsdna        621 -aaaca--acaatgt----tattgccgtaataggtgatgg
MTdxsdna        398 -accgc--aaccggcatgtggtcgcggtggtcggtgacgg
RSdxs1dna       423 -cacgg--gcgacgc----ggtggcggtgatcggcgacgg
RSdxs2dna       432 -cgtgg--gcgacac----gatcgccgtgatcggcgacgg
SPCCdxsdna      403 -gacta--ccgatgt----g-tcgctgtgattggtgatgg
ECdxsdna        431 -gcc----gca---c----cgtctgtgtcattggcgatgg
NMdxsdna        415 -gaccg--ccgcagc----g-tcgccatcatcggcgacgg
HIdxsdna        423 tgcaggtagaaaaac----agtatgcgtaatcggtgatgg
SSdxsdna        402 -gaagg--gccatgt----cgtcgccgtcatcggcggacg
HPdxsdna        396 -gctag--gcatgcc----catagctttattaggcgatgg

STdxsdna        637 cgcgatgagcgcgggcatggcctatgaggcgatgaacaac
CRdxsdna        672 cgccatcaccgggggtatggcctatgaggccatgaaccat
CJdxsdna        429 tgctttaagtgcgggtatggcctatgaggctttaaatgaa
PAdxsdna        480 tgcgctgaccgccggcatggccttcgaggcactcaaccac
LEdxsdna        654 tgccatgacagcaggtcaagcttatgaagccatgaataat
MTdxsdna        435 tgcgctcaccggcggtatgtgctgggaggcgctgaacaat
RSdxs1dna       456 ctcgatgtcggccggcatggccttcgaggcgctgaaccac
RSdxs2dna       465 ctccatcaccgcgggcatggcctacgaggcactgaaccac
SPCCdxsdna      435 atcgctcaccggtggcatggccttggaagccatcaaccac
ECdxsdna        459 cgcgattaccgcaggcatggcgtttgaagcgatgaatcac
NMdxsdna        447 cgcgatgacggcgggtcaggcgtttgaagccttgaactgc
HIdxsdna        459 cgcaattactgcgggaatggcatttgaggcattaaatcac
SSdxsdna        435 ggcgctgaccggcggcatggcctgggaggccctgaacaac
HPdxsdna        429 gagcattagtgcagggatttttatgaagccttaaacga-
```

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```
STdxsdna        677 gccgaggcc--gccgg--caa--c-cggc------t--gg
CRdxsdna        712 gcgggcttc--ctgga--caa--g-aaca------t--ga
CJdxsdna        469 ttgggtgat--tctaa--att--t-cctt------g--cg
PAdxsdna        520 gcctcggaa--gtcga--cgc--c-gaca------t--gc
LEdxsdna        694 gc--tggtt--acctg--gac--t-ctgaca----t--ga
MTdxsdna        475 atc---gcc--gcatc--ccg--c-cggc------c--gg
RSdxs1dna       496 ggcgggcac--ctgaa--gaa--c-cggg------t--ga
RSdxs2dna       505 gc--gggcc--atctgaacaa--g-cgcc------t--gt
SPCCdxsdna      475 gctggtcacttgccca--aaa--cacggc------t--gt
ECdxsdna        499 ------gcg--ggcga--tat--c-cgtcctgatat--gc
NMdxsdna        487 gc--gggcg--atatg--gat--g-tgga------tttgc
HIdxsdna        499 gcgggggc----attg--cat--a-caga------tatgt
SSdxsdna        475 atcgcggcc--gccaa--gga--c-cagc------c--gc
HPdxsdna        468 -actgggcg--atagg--aaatac-ccca------t--ga

STdxsdna        702 tggtgatcct---c---aacgacaac-gaca---tgtcga
CRdxsdna        737 ttgtgattct---g---aacgacaac-cagcaggtgtcgc
CJdxsdna        494 taatactttt---a---aatgataat-gaaa---tgagta
PAdxsdna        545 tggtgatcct---c---aacgacaac-gaca---tgtcga
LEdxsdna        719 ttgttatctt---a---aacgacaatagaca---agtttc
MTdxsdna        497 tgattatcgtggtc---aacgacaat-gggc---gcagct
RSdxs1dna       521 tcgtgatcct---g---aacgacaac-gaga---tgagca
RSdxs2dna       530 tcgtgatcct---g---aacgacaat-gaca---tgagca
SPCCdxsdna      503 tggtcgtgct---c---aacgacaat-gaca---tgtcga
ECdxsdna        524 tggtgattct---c---aacgacaat-gaaa---tgtcga
NMdxsdna        512 tggtcgtcct---c---aacgacaac-gaaa---tgtcga
HIdxsdna        524 tagttatttt---a---aatgataac-gaaa---tgtcta
SSdxsdna        500 tgatcatcgt---cgtcaacgacaac-gagc---gctcct
HPdxsdna        494 tcatgatttt---a---aacgataat-gaaa---tgagta

STdxsdna        732 tcgccccgccg----------------------gt---
CRdxsdna        770 tgcccacgcagtacaacaacaagaaccaggaccccgt---
CJdxsdna        524 tttcaaaacca----------------------at---
PAdxsdna        575 tctcgcacaac----------------------gt---
LEdxsdna        750 tttacctactg----------------------ctact
MTdxsdna        530 acgcgcccaca----------------------at---
RSdxs1dna       551 tcgcgccgccg----------------------gt---
RSdxs2dna       560 tcgcgccgccc----------------------gt---
SPCCdxsdna      533 tctcgcccaac----------------------gt---
ECdxsdna        554 tttccgaaaat----------------------gt---
NMdxsdna        542 tttcccccaac----------------------gt---
HIdxsdna        554 tttcagaaaac----------------------gt---
SSdxsdna        533 acgcgcccacc----------------------at---
HPdxsdna        524 tcagcacgcct----------------------at---
```

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```
STdxsdna        745 --gggcgggctttcggcctatcttgcgcgcctcatttcct
CRdxsdna        807 --gggcgccctgtccagcgccctggcgcgcctgcaggcca
CJdxsdna        537 --tggagcaatttcaaagtatctttctcaggctatggcaa
PAdxsdna        588 --cggcgggctctccaactacctggcgaagatcctctcca
LEdxsdna        766 ctggatgggccagttgctcctgttggagctctaagtagtg
MTdxsdna        543 --cgggggcgtcgccgaccatctggccacgctg-------
RSdxs1dna       564 --gggggcgctgtcgtcctatctctcgcggctc-tatgcg
RSdxs2dna       573 --gggggcgcttgcgcgctatctcgtgaatctc---tcct
SPCCdxsdna      546 --gggtgcgctctctcgctatct-----gaataagattcg
ECdxsdna        567 --cggcgcgctcaacaaccatctggcacagctgctttcc-
NMdxsdna        555 --cggtgcgttgcccaaataccttgccagc-----aacgt
HIdxsdna        567 --tggtgcattaaataatcatcttgcgcg---tattttct
SSdxsdna        546 --cggcggcctcgccaaccacctggccaccctgcgcacca
HPdxsdna        537 --tggagccttatccaaagcccttagccagctga--tgaa

STdxsdna        783 c--gtc--------------cga-ata----t--------
CRdxsdna        845 a--ccg--------------gcc-cct----g--------
CJdxsdna        575 c--gca--------------gtt-tta----t--------
PAdxsdna        626 g--ccg--------------cac-cta----t--------
LEdxsdna        806 c--tttgagcaggttacagtcta-ataggcct--------
MTdxsdna        574 c--ggc--------------tgc-a---------------
RSdxs1dna       601 g--gcg--------------cgc-cgt----t--------
RSdxs2dna       608 c--gaa--------------ggc-gcc----c--------
SPCCdxsdna      579 g--gtt--------------ag------------------
ECdxsdna        604 g--gta--------------agc-ttt----a--------
NMdxsdna        588 c--gtg--------------cgcgata----tg-------
HIdxsdna        602 ctggct--------------ctc-ttt----a--------
SSdxsdna        584 c--cga--------------cgg-cta----cgagaaggt
HPdxsdna        573 a--ggc--------------ccg-ttt----t--------

STdxsdna        794 -ctcggc---c--tgc-gcga-gc---tcgcc--------
CRdxsdna        856 -cgcgag---c--tgc-gcga-ga---ttgcc--------
CJdxsdna        586 -caaagt---t--tta-aaaa-gcgtattgct--------
PAdxsdna        637 -agcagc---a--tgc-gcga-gg---gcagc--------
LEdxsdna        835 -ctcagagaac--taa-gaga-ag---tcgca--------
MTdxsdna        582 -gccggc---c--tac-gag-----------c--------
RSdxs1dna       612 -ccagga---c--ttc-aaggcgg---ccgcc--------
RSdxs2dna       619 -ttcgccacgc--tgc-gcgc-gg---ccgcc--------
SPCCdxsdna      585 --tgagc---cgatgc-agtt-gc---tcacc--------
ECdxsdna        615 -ctcttca--c--tgc-gcga------------------
NMdxsdna        601 -cacgga---c--tgttgagt-ac---cgtca--------
HIdxsdna        615 -ctctacg--c--ttc-gtga-tg---gcagt--------
SSdxsdna        603 cctcgcc---t--ggg-gcaa-gg---acgtc--------
HPdxsdna        584 -accagt---c--ttt-ccgc-tc---taaagttaaaaaa
```

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```
STdxsdna        815 -----aagcg----------cttcac-----cc-------
CRdxsdna        877 -----aaggg----------cgtgac-----ca-------
CJdxsdna        610 -----aaaat----------gtt-----------------
PAdxsdna        658 -----aagaa----------ggt-----------------
LEdxsdna        859 -----aaggg----------agttac-----ta-------
MTdxsdna        596 -----aggcg----------ctggagacgggcc-------
RSdxs1dna       634 -----aagggagcgctcgggcttctg-----cc-------
RSdxs2dna       643 -----gacgg----------gctcga-----gg-------
SPCCdxsdna      607 -----gatgg----------tttgac-----ccaggggat
ECdxsdna        630 -----aggcg----------ggaaaa-----aa-------
NMdxsdna        623 -----aagcg----------c-aaac-----gg-------
HIdxsdna        637 -----aaaaa----------aatc-----------------
SSdxsdna        625 -----ctgct----------gcgtac-----cc-------
HPdxsdna        613 atcttaagca----------ccttac-----ct-------

STdxsdna        828 gcaag------ctttcg----cgccgc---c--tcaccgc
CRdxsdna        890 agcag------ctgcct----gacgtt---g--tccagaa
CJdxsdna        618 ggata------tatt----------gc---c--tgatagt
PAdxsdna        666 ----g------ctctcg----cgcctg---c--ccggggc
LEdxsdna        872 agcag------attggt----ggtcct---a--tgcatga
MTdxsdna        614 gcgac------ctggtg----cgc-gc---g--gtgccgc
RSdxs1dna       657 cgaac------cgttcc----aggagggcgc--gcgccgc
RSdxs2dna       656 cctcg------ctgccg----gggccg---c--tccgcga
SPCCdxsdna      627 gcaacaaattcccttcgtcggcggcgc---cattacccaa
ECdxsdna        643 gtttt------ctctgg----cgtgcc---g--ccaatta
NMdxsdna        635 gcaag------gtatta----gacaaa---a--tacccgg
HIdxsdna        646 -cttg------ataaag----ttcctc---caatcaaaaa
SSdxsdna        638 ccatc------gtcggc----cacccc---c--tctacga
HPdxsdna        631 gaaag------cgt-----------ga---a--ttactta

STdxsdna        853 --g-gc----agccggcaaggcg----g-----aggaa--
CRdxsdna        915 --g-gc----aactgctaagatt----g-----acgag--
CJdxsdna        637 ----gc----tacttatatggcc----a-----agcgt--
PAdxsdna        687 ctg-gg----agatcgcccggcgcaccg-----aggaa--
LEdxsdna        897 --g-ct----tgctgcaaaagtt----g-----atgaa--
MTdxsdna        638 --ttgt----cggcggtctgtgg----t-----ttcga--
RSdxs1dna       685 --g-cc----a--aggagatgct----g-----aaga---
RSdxs2dna       681 --c-gg----ggcgcgccgggcg----c-----gccag--
SPCCdxsdna      664 --g-gctttgagccggttaag-g----a-----aggca--
ECdxsdna        668 --a-ag----agctgctcaaacgcaccg-----aagaa--
NMdxsdna        660 --c-gc----gatggagtttgcc----c-----aaaaa--
HIdxsdna        672 --t-tt----tatgaaaaaaacc----g-----aagaaca
SSdxsdna        663 --g-gc------cctgcacggcg----ccaagaagggc--
HPdxsdna        649 --g-cg----agtcg----tttt----g-----aagaa--
```

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```
STdxsdna        875 t--------tcgcccgcggcatg----g----cg------
CRdxsdna        937 t--------atgctcgcggcatgatcag----cggc----
CJdxsdna        658 t--------ttgaagagagtttt----a----aacttatt
PAdxsdna        715 t--------acgccaagggcatg----c----tg------
LEdxsdna        919 t--------atgctcgtggcatg----a----tt------
MTdxsdna        661 t--------tcctgcacagcgtc----a----ag------
RSdxs1dna       704 ----------------gcgtc----a----cc------
RSdxs2dna       703 c--------tcgtgaccgggatg----c----cg------
SPCCdxsdna      689 tgaagcgcctctcctacagcaag----------------
ECdxsdna        694 c--------atattaaaggcatg----g----ta------
NMdxsdna        682 g--------tcgaacataaaatc----a----aa------
HIdxsdna        696 t--------atgaaaggtgtaat----gttttcg------
SSdxsdna        688 t--------tcaaggacgccttc----g----cc------
HPdxsdna        667 t--------ctttcaagctcat----------c------

STdxsdna        893 a------ccg----------gcggcacg----------
CRdxsdna        961 a------ctg----------gctccacg----------
CJdxsdna        682 a------ccc----------ctgggctt----------
PAdxsdna        733 g------tcc----------ccggcacc----------
LEdxsdna        937 agtggttctg----------gatcaaca----------
MTdxsdna        679 g------ccg----------gcatcaaggactcgctgtc
RSdxs1dna       712 g------tcg----------gcggcacg----------
RSdxs2dna       721 g------gcg----------ggggcacg----------
SPCCdxsdna      712 ---------a----------ttggggcg----------
ECdxsdna        712 g------tgc----------ctggcacg----------
NMdxsdna        700 a------cccttgccgaagaagccgaaca----------
HIdxsdna        718 c------cag----------aaagtaca----------
SSdxsdna        706 c------cgc----------agggca------------
HPdxsdna        682 a------ccc----------cgggcgtg----------

STdxsdna        905 ----------c--------------------tgttcgagga
CRdxsdna        973 ----------c--------------------tgtttgagga
CJdxsdna        694 ----------t--------------------tgtttgaaga
PAdxsdna        745 ----------c--------------------tgttcgagga
LEdxsdna        955 ----------t--------------------tgtttgaaga
MTdxsdna        702 gccgcagttgc--------------------tgttcaccga
RSdxs1dna       724 ----------c--------------------tcttcgagga
RSdxs2dna       733 ----------c--------------------tcttcgagga
SPCCdxsdna      721 ----------g--------------------tctttgaaga
ECdxsdna        724 ----------t--------------------tgtttgaaga
NMdxsdna        723 ----------cgccaaacagtcactgtctttgtttgaaaa
HIdxsdna        730 ----------t--------------------tatttgaaga
SSdxsdna        716 ------------------------------tgttcgagga
HPdxsdna        694 ----------t--------------------ttttgaaga
```

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```
STdxsdna        916 acttggcttctattatgtcggcccgatcgacggccacaat
CRdxsdna        984 gctgggcctgtactacatcggccctgtggacggccacaac
CJdxsdna        705 attagggcttgaatatatagggcctattgatggacataat
PAdxsdna        756 gctcggctggaattacatcggcccgatcgacggccacgac
LEdxsdna        966 acttggactttactatattggtcctgtggatggtcacaac
MTdxsdna        723 cctcgggttgaagtacgtcggcccggtcgacggcca---t
RSdxs1dna       735 gctgggtttctcctatgtcggcccgatcgacgggcacgat
RSdxs2dna       744 gctgggcttcacctatgtcggccccatcgacggccacgac
SPCCdxsdna      732 gctgggcttcacctacatggggccagtggatggtcacaac
ECdxsdna        735 gctgggctttaactacatcggcccggtggacggtcacgat
NMdxsdna        753 cttcggcttccgctataccggccccgtggacggacacaac
HIdxsdna        741 actcggttttaactatattggcccagtggatgggcataac
SSdxsdna        726 cctgggcctgaagtacgtcggccccatcgacgggcacgac
HPdxsdna        705 attaggcattaactatatagggcctattaatgggc-----

STdxsdna        956 ctcgagcatctgatcccggtgctggagaatgtcc-g----
CRdxsdna       1024 ctggacgacctcatcgccgtgctcagcgaggtgc-g----
CJdxsdna        745 ttaggtgaaattat-----ttctgcattaaaacaag----
PAdxsdna        796 ctgccgaccctggtggctaccctgcgcaacatgc-g----
LEdxsdna       1006 attgatgatctaattgcgattctcaaagaggtta-gaagt
MTdxsdna        760 gacgag---------cgggcggtggaggtcgcgc-t----
RSdxs1dna       775 ctcgaccagcttctgccggtgctgcggaccgtca-a----
RSdxs2dna       784 atggaggcgctcctccagacgctgcgcgcggcgc-g----
SPCCdxsdna      772 cttgaagaactgatc---------gccaccttcc-g----
ECdxsdna        775 gtgctggggcttatcaccacgctaaagaacatgc-g----
NMdxsdna        793 gtcgaaaatctggtcgatgtattggaagacctgc-g----
HIdxsdna        781 attgatgaattagtggctacgcttacgaatatgc-g----
SSdxsdna        766 atcggcgcggtcgagtccgcgctgc------gcc-g----
HPdxsdna        740 ----atgatttgagcgcgattattgaaaccttaa-a----

STdxsdna        991 -c---gaca---gcga-gc---a---g-------ggc---
CRdxsdna       1059 -c---agcg---ccga-ga---ccgtg-------ggc---
CJdxsdna        776 -c---aaaa---gctatgc---a---a-------aag---
PAdxsdna        831 -c---gaca------t-ga---a---g-------ggc---
LEdxsdna       1045 ac---taaa---ac-a-ac---a---g-------gtc---
MTdxsdna        786 -g---cgca---gcgc-gc---g---g-------cgcttc
RSdxs1dna       810 -g---cagc---gggc-gc---a---t-------gcg---
RSdxs2dna       819 -g---gccc---g-ga-cc---ac--g-------ggg---
SPCCdxsdna      798 -c---ga-a---gcgc-ac---a---aacacaccgga---
ECdxsdna        810 -c---ga------cct-ga---a---a-------ggc---
NMdxsdna        828 -c---ggac---gc----a---a---a-------ggc---
HIdxsdna        816 -------ta---atct-ga---a---a-------ggc---
SSdxsdna        795 -c---gccaagcgctt-cc---a---c-------ggg---
HPdxsdna        771 -attagcca---aaga-gcttaa---a-------gag---
```

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```
STdxsdna      1007 ------ccgatcctgatccatgtcgtgaccaagaagggca
CRdxsdna      1078 ------ccggtgctggtgcacgtggtaacggagaagggcc
CJdxsdna       793 ------ccttgtgtgatacatgctcaaaccataaagggta
PAdxsdna       844 ------ccgcagttcctccatgtggtgaccaagaaaggca
LEdxsdna      1061 ------cag-tactgatccatgttgtcactgagaaaggca
MTdxsdna       805 ggtgcaccggtgatcgtgcacgtcgtcacccgcaagggca
RSdxs1dna      826 ------ccggtgctgatccatgtcatcaccaagaagggca
RSdxs2dna      835 ------ccggtgctcatccatgtggtcacgaagaagggca
SPCCdxsdna     820 ------ccagtactcgtccacgttgccacaaccaagggta
ECdxsdna       823 ------ccgcagttcctgcatatcatgaccaaaaaaggtc
NMdxsdna       841 ------ccgcagcttctgcacgtcatcaccaaaaagggca
HIdxsdna       829 ------ccacaatttttgcatataaaaacgaaaaaaggta
SSdxsdna       814 ------ccggtgctggtgcactgcctcaccgtcaagggcc
HPdxsdna       793 ------ccggtgctaatccatgcgcaaaccttaaagggca

STdxsdna      1041 agggctatgccccggccgaagcg---gcggcggacaagta
CRdxsdna      1112 gcggctacctgcccgccgagacg---gcgcaggacaagat
CJdxsdna       827 aaggctatgctttagctgaagga---aaacatgctaaatg
PAdxsdna       878 agggcttcgccccggccgaactg---gatccgatcggcta
LEdxsdna      1094 gaggttatccatatgctgagaga---gctgcagataagta
MTdxsdna       845 tgggctacccgccggccga---------ggccgac-----
RSdxs1dna      860 ggggctatgctccggccgaggcc---gcgcgcgaccgtgg
RSdxs2dna      869 agggttacgcccccgccgagaat---gcccccgacaagta
SPCCdxsdna     854 agggctatccctacgctgaagaa---gatcaggttggcta
ECdxsdna       857 gtggttatgaaccggcagaaaaa---gacccgatcacttt
NMdxsdna       875 acggctacaaactcgccgaaaac---gatcccgtcaaata
HIdxsdna       863 aaggatacgcacccgcagaaaaa---gatccgattggttt
SSdxsdna       848 gcggctacgaacccgccctcgcccacgaggaggaccactt
HPdxsdna       827 aaggctataagatcgctgaaggg---cgctatgaaaaatg

STdxsdna      1078 tcacggcgtccagaag--tt--cgacgt----gatc-acc
CRdxsdna      1149 gcacggtgtggtcaag--tt--cgaccc----ccgc-acc
CJdxsdna       864 gcacggggtgggagcc--tt--tgatat----agat-agt
PAdxsdna       915 ccacgcgatcaccaag--ct--gga--------agc-tcc
LEdxsdna      1131 tcatggagttgccaag--tt--tgatcc----agca-aca
MTdxsdna       871 -caggccgagcagatgcatt--ccacggtcccgatcgatc
RSdxs1dna      897 ccatgccacgaacaag--tt--caacgt----cctg-acc
RSdxs2dna      906 tcacggggtgaacaag--tt--cgaccc----cgtc-acg
SPCCdxsdna     891 tcatgcccaaaatccc--tt--tgatct----ggcg-aca
ECdxsdna       894 ccacgccgtgcctaaa--tt--tgatcc----ctcc-agc
NMdxsdna       912 ccacgccgtcgccaac--ctgcctaaag----aaag-cgc
HIdxsdna       900 ccacggtgtacctaaa--tt--tgatcc----aatc-agt
SSdxsdna       888 ccacaccgtcggcgtg--at--ggaccc----gctc-acc
HPdxsdna       864 gcatggggtggggcct--tt--tgattt----ggat-acc
```

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```
STdxsdna      1109 ggggcacaggcc-----aaggcaccc------ccgggcc-
CRdxsdna      1180 ggcaagcaggtg-----caggccaag------acgaagg-
CJdxsdna       895 ggagagagtgtt-----aaaaaaagt------gatacta-
PAdxsdna       942 cggcagtgcgcc-----gaagaagac------c--ggcg-
LEdxsdna      1162 ggaaagcaattc-----aaag----c------cagtgcca
MTdxsdna       908 cggccaccggac-----aagccacca------aggtggc-
RSdxs1dna      928 ggcgcgcaggtg-----aagccggtc------tcgaacg-
RSdxs2dna      937 ggcgagcagaag-----aagtcggtg------gccaacg-
SPCCdxsdna     922 ggg---aaggct-----aaaccagcttcaaaaccgaagc-
ECdxsdna       925 ggttgtttgccg-----aaaagtagc------ggcggtt-
NMdxsdna       945 ggcgcaaatgccgtctgaaaaagaac------ccaagcc-
HIdxsdna       931 ggcgaattgccc-----aa---aaac------aatagta-
SSdxsdna       919 tgtg---agccc-----ctctcgccc------accgacg-
HPdxsdna       895 gg---cttgtct-----aaaaaatcc------aaaag---

STdxsdna      1137 ---cgccc--gc--------ctat-----accaaggtgtt
CRdxsdna      1208 ---ccatg--tc--------gtac-----acgaactactt
CJdxsdna       923 ---aaaaa--tc--------tgct-----actgaaatttt
PAdxsdna       968 ---gaccc--aa--------gtat-----tccagcgtctt
LEdxsdna      1187 agacacag--tc--------ctat-----acaacatattt
MTdxsdna       936 ---cggcccagg--------ctgg-----acggcgacctt
RSdxs1dna      956 ---ccccc--tc--------ctat-----accaaggtctt
RSdxs2dna      965 ---cgccg--aa--------ctac-----accaaggtctt
SPCCdxsdna     953 ---cgcct--ag--------ctat-----tccaaagtgtt
ECdxsdna       953 ---tgccg--ag--------ctat-----tcaaaaatctt
NMdxsdna       978 ---cgccg--ccaaaccgacctat-----acccaagtgtt
HIdxsdna       956 ---aacca--ac--------ttat-----tcgaaaatttt
SSdxsdna       944 ---gcccg--tc--------ctgg-----acctcggtgtt
HPdxsdna       918 ---cgcaa--tc--------ttatcgcccactgaagcgta

STdxsdna      1159 cgccgatgcgctgctcgc-cgaagcgg-agcgtgatgcgt
CRdxsdna      1230 cgcggacgcgctgacggc-ggaggcgg-agcgcgacagcc
CJdxsdna       945 ttctaagaatttgcttga-tttagcct-caaaatatgaaa
PAdxsdna       990 cggccagtggctgtgcga-catggccg-cccaggacgcg-
LEdxsdna      1212 tgccgaggctttaattgc-agaagcag-aagcagataaag
MTdxsdna       960 ctctgatgcacttatcgg-ctacgc-----ccagaaacgc
RSdxs1dna      978 cgcccagagcctcatcaa-ggaggccg-aggtcgacgagc
RSdxs2dna      987 cggctccaccctgaccga-ggaggccg-cgcgcgatccgc
SPCCdxsdna     975 tggccaaaccctgacgac-cttggcca-agagcgat-cgc
ECdxsdna       975 tggcga----ctggttgtgcgaaacggcagcgaaagacaa
NMdxsdna      1008 cggcaaatggctgtgcga-ccgggcgg-cggcagattc--
HIdxsdna       978 tggcgattggctatgtga-aatggcag-aaaaagatgcca
SSdxsdna       966 cggcgacgagatcgt--a-cggatcgg-cgcggagcgcga
HPdxsdna       945 ttctaacacccttttaga-attagcta-aaaaagatgaaa
```

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```
STdxsdna      1197 cgg----tctg-c--gcg--atcaccgcggcgatgccctc
CRdxsdna      1268 gca----tcgt-g--gcg--gtgcacgcggccatggcggg
CJdxsdna       983 ata----ttgt-t--ggg--gttacggcggctatgccaag
PAdxsdna      1027 -cg----cctg-c--tcggcatcaccccggcgatgaagga
LEdxsdna      1250 aca----ttgt-t--gca--atccatgctgccatgggggg
MTdxsdna       994 cgtgacatcgt-g--gcc--attaccgcggccatgccggg
RSdxs1dna     1016 gga----tctg-c--gcg--gtgacggccgccatgccgga
RSdxs2dna     1025 gca----tcgt-g--gcg--atcaccgccgctatgccctc
SPCCdxsdna    1012 cgc----attgtc--ggg--attacggctgcgatggcgac
ECdxsdna      1011 caa----gctg-atggcg--attactccggcgatgcgtga
NMdxsdna      1044 ccg----actg-gttgcg--attacccccgccatgcgcga
HIdxsdna      1016 aaa----ttat-a--ggt--atcacacctgcaatgcgtga
SSdxsdna      1002 ggaca--tcgt-c--gcg--atcaccgccgcgatgctc--
HPdxsdna       983 aaa----tcgt-a--ggc--gtaaccgcggcgatgcctag

STdxsdna      1228 gggcacc-----gggctcg-acaagttccaggcgacg--t
CRdxsdna      1299 cggcacc-----ggcctgt-accggttcgagaagaag--t
CJdxsdna      1014 tggaaca-----ggtcttg-ataagcttatagaaaaa--t
PAdxsdna      1059 aggttcc-----gacctgg-tggcctt-cagcgaacg--t
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MTdxsdna      1029 ccccacc-----gggctga-ccgcgttcgggcagcgc--t
RSdxs1dna     1047 cgggacg-----gggctca-acctcttcggcgagcgg--t
RSdxs2dna     1056 gggcacc-----ggcgtcg-acatcatgcagaagcgt--t
SPCCdxsdna    1044 aggcacc-----ggcttgg-acattctccagaaggcg--c
ECdxsdna      1044 aggttcc-----ggcatgg-tcgagttttcacgtaaa--t
NMdxsdna      1077 gggcagc-----ggcttgg-ttgagtttga-acaacga-t
HIdxsdna      1047 gggttca-----ggtatggtagaatttc--ccaacgc-t
SSdxsdna      1033 ---cacccggtggggctcg-ccaggttc--gccgaccgct
HPdxsdna      1014 cggcaca-----ggattag-acaaactcattgacgct--t

STdxsdna      1260 tccccg-atc-gcaccttcgacgtcgctatcgccgagcag
CRdxsdna      1331 tcccgg-acc-gcacctttgacgtgggcattgcggagcag
CJdxsdna      1046 atccaa-atc-gtttttgggatgtggctattgcagaacag
PAdxsdna      1090 tatccggaac-gctacttcgacgtcgccatcgccgaacag
LEdxsdna      1312 ttccca-acaaggtgttttgatgttggaatagcagaacaa
MTdxsdna      1061 tcccgg-atc-gattgttcgacgtcgggatcgccgagcaa
RSdxs1dna     1079 ttccga-agc-gcaccttcgatgtgggcatcgcggaacag
RSdxs2dna     1088 tcccga-acc-gcgtcttcgacgtgggcatcgccgagcag
SPCCdxsdna    1076 tgccga-agc-aatacatcgatgttggcattgccgaacag
ECdxsdna      1076 tcccgg-atc-gctacttcgacgtggcaattgccgagcaa
NMdxsdna      1109 tccccg-acc-gctatttcgatgtcggcatcgccgagcag
HIdxsdna      1079 tcccaa-aac-aatattttgacgtagcgattgcagaacag
SSdxsdna      1067 tcccgg-acc-gggtctgggacgtcggcatcgccgagcag
HPdxsdna      1046 accctt-tgc-gctttttttgatgtcgctatcgctgagcaa
```

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```
STdxsdna      1298 cacgcggtcacct-tcgcagcgggccttgccgcgcagggg
CRdxsdna      1369 cacgccgtgacct-ttgctgccggcctggcgtgcgagggc
CJdxsdna      1084 catgcagtaactt-ctatggccgctatggcaaaagaagga
PAdxsdna      1129 catgccgtgaccc-tggccgccggcatggcctgcgagggc
LEdxsdna      1351 catgcagtaacct-ttgctgctggattggcttgtgaaggc
MTdxsdna      1099 cacgcgatgacgt-cggcggccgggttggcgatgggtggg
RSdxs1dna     1117 catgcggtgacct-tctcggcggcgcttgcggcaggcggc
RSdxs2dna     1126 catgccgtgacct-tcgcggccggcctcgccggggccggg
SPCCdxsdna    1114 cacgccgtggtgc-tagctgccggtatggcctgcgatggc
ECdxsdna      1114 cacgcggtgacct-ttgctgcgggtctggcgattggtggg
NMdxsdna      1147 cacgccgttacct-ttgccggcggtttggcttgcgaaggg
HIdxsdna      1117 cacgctgtcacgt-ttgccacaggacttgcaattggcgga
SSdxsdna      1105 cacgcggccgtgt-ccgcggccgggctcgccaccggcgga
HPdxsdna      1084 cacgctttaacttctagcagc--gctatggctaaagaggg

STdxsdna      1337 atgcggccgttctgcgcg-atctactcgaccttcctgcag
CRdxsdna      1408 ctggtgcccttctgcacc-atctacagtaccttcatgcag
CJdxsdna      1123 tttaaacctttattgca-atatatagcacctttttgcag
PAdxsdna      1168 atgaagccggtggtagcg-atctactcgaccttcctccag
LEdxsdna      1390 attaaacctttctgtgca-atctattcgtctttcatgcag
MTdxsdna      1138 ctgcaccccgtggtggcg-atctactcgacgttcctgaac
RSdxs1dna     1156 atgcggcccttctgcgcc-atctattccaccttcctccag
RSdxs2dna     1165 atgaagcccttctgcgcg-atctattcctcgttcctgcaa
SPCCdxsdna    1153 atgcgtccggtggtggca-atctattccaccttcctgcag
ECdxsdna      1153 tacaaacccattgtcgcg-atttactccactttcctgcaa
NMdxsdna      1186 atgaagcccgtcgtggcg-atttattccacctttttacaa
HIdxsdna      1156 tataaacctgtcgtcgca-atttactcgacatttttacaa
SSdxsdna      1144 ctgcacccggtcgtcgcc-gtctacgccaccttcctcaac
HPdxsdna      1122 gtttaaacctttttgtgagcatctattctacttttttgcag

STdxsdna      1376 cgcgcctacgaccaggtcgtccacgacgtcgcgatccaga
CRdxsdna      1447 cgcggttacgaccagatcgtgcacgacgtgtccctgcaga
CJdxsdna      1162 cgtgcttatgatcaagtgatccatgattgtgcgattatga
PAdxsdna      1207 cgcgcctacgaccagttgatccatgacgtcgccgtgcagc
LEdxsdna      1429 agggcttatgaccaggtagtgcatgacgttgatttgcaaa
MTdxsdna      1177 cgggcgttcgaccagatcatgatggatgtggcgctgcaca
RSdxs1dna     1195 cgcggctacgaccagatcgtgcatgacgtggcaatccagc
RSdxs2dna     1204 cggggttacgaccagatcgcccatgacgtggcgctgcaga
SPCCdxsdna    1192 cgggcctttgatcaagtcatccacgacgtttgtatccaaa
ECdxsdna      1192 cgcgcctatgatcaggtgctgcatgacgtggcgattcaaa
NMdxsdna      1225 cgcgcctacgaccaactggtgcacgacatcgccctgcaaa
HIdxsdna      1195 cgtgcttacgatcaattaattcacgatgttgccattcaaa
SSdxsdna      1183 cgcgccttcgaccagctcctgatggacgtcgc---cctgc
HPdxsdna      1162 agggcttatgattctattgtgcatgacgcttgtatttcta
```

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```
STdxsdna      1416 acc--tgccg-gtccgcttcgcgatcgaccgcgcgggcct
CRdxsdna      1487 agc--tgcct-gtgcgcttcgctatggaccgcgctggcct
CJdxsdna      1202 att--taaat-gtggtttttgctatggatagggcagggat
PAdxsdna      1247 acc--tcgac-gtgctgttcgccatcgaccgcgccggcct
LEdxsdna      1469 agc--tgccc-gtgaggtttgcaatggacagagcaggtct
MTdxsdna      1217 agc--tgccg-gtcaccatggtgctggaccgtgccgggat
RSdxs1dna     1235 gcc--tgccg-gtgcgctttgccatcgaccgcgccggcct
RSdxs2dna     1244 acc--ttccc-gtccgcttcgtgatcgaccgggcggggct
SPCCdxsdna    1232 agc--tgccc-gtcttcttctgcctcgatcgcgcggggat
ECdxsdna      1232 agc--ttccg-gtcctgttcgccatcgaccgcgcgggcat
NMdxsdna      1265 acc--tgccc-gttttgtttgccgtcgaccgcgcgggcat
HIdxsdna      1235 atc--tccct-gtgctatttgcaattgatcgagcagggat
SSdxsdna      1220 accgctgcggtgtgaccttcgtcctggaccgggccggcgt
HPdxsdna      1202 gct--tgccg-attaaattagccattgacagggctgggat

STdxsdna      1453 ggtcggtgccgacggcgcgacccatgccggcagcttcgac
CRdxsdna      1524 ggtgggcgctgacggctccacgcactgcggcgccttcgac
CJdxsdna      1239 agtaggcgaagatggggagacgcatcaaggtgtttttgat
PAdxsdna      1284 ggtcggcgaggacggcccgacccacgccggtagcttcgac
LEdxsdna      1506 tgttggagcagatggtccaacacattgtggtgcatttgat
MTdxsdna      1254 caccggtagcgacggcgccagccacaacggaatgtgggac
RSdxs1dna     1272 cgtgggggcggacggcgccacccatgcgggctcgttcgat
RSdxs2dna     1281 cgtgggggccgatggcgcgacccatgcgggggccttcgac
SPCCdxsdna    1269 agttggcgcggatggcccgactcaccaaggcatgtacgac
ECdxsdna      1269 tgttggtgctgacggtcaaacccatcagggtgcttttgat
NMdxsdna      1302 cgtcggcgcggacggcccgacccatgccggtttgtacgat
HIdxsdna      1272 agttggtgcagatggggctacacatcaaggtgcattcgat
SSdxsdna      1260 cacgggcgtcgacggcgcctcgcacaacggcatgtgggac
HPdxsdna      1239 tgtgggcgaagatggcgagacgcaccaagggcttttagac

STdxsdna      1493 gtgacctatctcgccagcctgcccaatttcgtggtgatgg
CRdxsdna      1564 gtgacgttcatggcgtcgctgccgcacatgatcaccatgg
CJdxsdna      1279 cttagtttttagctcctttgccaaatttcactcttttag
PAdxsdna      1324 atctcctacctgcgctgcatccccggcatgctggtgatga
LEdxsdna      1546 gttacttacatggcatgtcttcctaacatggttgtaatgg
MTdxsdna      1294 ttgtcgatgctgggtatcgtgcccggcatccgggtggcag
RSdxs1dna     1312 gtggccttcctgtcgaacctgcccggcatcgtggtgatgg
RSdxs2dna     1321 gttggcttcatcacttcgctgcccaacatgaccgtgatgg
SPCCdxsdna    1309 attgcttacctgcggctgattcccaacatggtgctgatgg
ECdxsdna      1309 ctctcttacctgcgctgcataccggaaatggtcattatga
NMdxsdna      1342 ttaagcttttgcgctgcattccgaat---atgattgtcg
HIdxsdna      1312 attagctttatgcgttgcattccaaatatgatcattatga
SSdxsdna      1300 atgtccgtcctccaggtcgtgccc---ggcctcaggatcg
HPdxsdna      1279 gtgtcgtatttgcgctctatccctaa---catggtcattt
```

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```
STdxsdna      1533 cggccgcggacgaggtcgag-ctcgtccacatg--accca
CRdxsdna      1604 ctccctcgaacgaggcggag-ctcatcaacatg--gtggc
CJdxsdna      1319 c-----cccaagagat--------gaacaaatg--atgca
PAdxsdna      1364 cccccagcgacgaggacgag-ctgcgcaagctg--ctcac
LEdxsdna      1586 ctccttctgatgaagcggag-ctatttcacatggtagcaa
MTdxsdna      1334 cgcccagagacg-----cca-cccggttgcgtg--aagaa
RSdxs1dna     1352 ccgccgccgacgaggccgag-ctcgtccatatg--gtagc
RSdxs2dna     1361 ccgcggccgacgaggccgag-ctcatccacatg--atcgc
SPCCdxsdna    1349 caccgaaagatgaggccgaa-c---tgcagcgg--atgct
ECdxsdna      1349 ccccgagcgatgaaaacgaa-tgtcgccagatg--ctcta
NMdxsdna      1379 ccgcgccgagcgatgaaaat-gaatgccgcctg--ctgct
HIdxsdna      1352 cgccgagtgatgaaaatgaa-tgccgtcaaatg--ctcta
SSdxsdna      1337 ccgccccgcgcgacgccgac-cacgtgcgcgcc--cagct
HPdxsdna      1316 ttgccccacgagacaatgagactttaaaaaacg--ccgtg

STdxsdna      1570 ca-cg---gcg--g--cga--tg--cacg-------acag
CRdxsdna      1641 ca-cctgcgcc--g--cca--tc--gacg-------ac--
CJdxsdna      1344 aa-at---ata--a--tgg--ag--tatgcttatttacat
PAdxsdna      1401 ca-c-----cg--g--ctacctg--ttcg-------a---
LEdxsdna      1625 ctgct---gcc--g--cca--tt--gatg-------aca-
MTdxsdna      1366 ct-cg---gcgagg--cgc--tc--gacgtcg----acga
RSdxs1dna     1389 ca-cc---gcc--g--ccg--cc--catg-------acga
RSdxs2dna     1398 ca-cc---gcc--g--tgg--cc--ttcg-------gcga
SPCCdxsdna    1383 ag-tg---acg--g--gta--tt--gaat-------acga
ECdxsdna      1386 ta-c-----cg--g--cta--t---cact-------ataa
NMdxsdna      1416 tt-cg---acc--t--gct--at--cagg-------caga
HIdxsdna      1389 ta-ca---ggt--tatcaa--tg--tgga-------aaac
SSdxsdna      1374 gc-gg---gag--g--cgg--tc--gccg-------tgga
HPdxsdna      1354 cg-tt---ttg--c--caa--tgaacacg-------attc

STdxsdna      1591 c--g---gcccgatcgcgctgc-gctatccacgcggcaac
CRdxsdna      1663 -------gcgccctcgtgcttccgcttcccccgcggcaac
CJdxsdna      1372 caag---gacctattgctttgc-gttatcctag-----ag
PAdxsdna      1419 t--g---gcccggccgcggtgc-gctatccgcgcggcagc
LEdxsdna      1646 -------gaccaagttgtttta-gatacccaagaggaaat
MTdxsdna      1392 c--g---gcccgacggcgttac-ggttccc------caaa
RSdxs1dna     1410 a--g---ggcccatcgccttcc-gctatccgcgcggcgac
RSdxs2dna     1419 g--g---gccccatcgccttcc-gcttcccgcggggcgag
SPCCdxsdna    1404 c--g---gcccgatcgccatgc-gtttcccgcgcgggaat
ECdxsdna      1404 c--gatggcccgtcagcggtgc-gctacccgcgtggcaac
NMdxsdna      1437 c--g---cgcccgccgccgtcc-gctatccgcgcggcacg
HIdxsdna      1412 c--t---gc-----ggcagtgc-gctaccctcgcggaaat
SSdxsdna      1395 c--g---acgcgccgacgctg-----atccgcttcccgaa
HPdxsdna      1377 a--a---gcccttgcgcgttcc-gatacc-------ctag
```

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```
STdxsdna        1625 ggcg---tcggactggc-gctgccc--------------
CRdxsdna        1696 ggcc---tgggcctgga-cctggccgcctacggcatcagc
CJdxsdna        1403 ggag---ttttattttg-gataaag--------------
PAdxsdna        1453 ggcc---ccaaccatcc-gatcgat--------------
LEdxsdna        1678 ggga---tcggtgtaga-gcttccg--------------
MTdxsdna        1420 ggtgatgtgggagaaga-tatttc---------------
RSdxs1dna       1444 ggcg---tgggggtcga-ggtgccg--------------
RSdxs2dna       1453 gggg---tgggcgtcga-gatgccc--------------
SPCCdxsdna      1438 ggta---ttggcgtacc-cctgccggaag----------
ECdxsdna        1441 gcgg---tcggcgtgga-actg-----------------
NMdxsdna        1471 ggta---cgggcgtgcc-ggtttca--------------
HIdxsdna        1441 gccg---ttggtgtaaa-act---t--------------
SSdxsdna        1425 ggag---tccgtcggcccgcggatc--------------
HPdxsdna        1404 gggg---tcg--tttgc-gttaaaa--------------

STdxsdna        1646 aa-gg---t--tccggag-----c------ggctg-----
CRdxsdna        1732 aa-gg---a--cctgaag-----g------gtgtgcccct
CJdxsdna        1424 aa-tt---taatccttgt-----g------agata-----
PAdxsdna        1474 cc-gg---a--cctgcaa-----c------cggtg-----
LEdxsdna        1699 gctgg---a--aacaaaggaattc------ctctt-----
MTdxsdna        1443 ---gg---c--tttggag-----c------ggcgt-----
RSdxs1dna       1465 gt-ga---a--gggcgtg-----c------cgctc-----
RSdxs2dna       1474 ga-gc---g--cgggacg-----g------tgctg-----
SPCCdxsdna      1463 aa-gg---c--tg-ggag-----t------cgctc-----
ECdxsdna        1459 ac-gc---c--gctggaa-----a------aacta-----
NMdxsdna        1492 ga-cg---g--catggaa-----a------ccgtg-----
HIdxsdna        1459 ac-tc---c--tttagaa-----a------tgctt-----
SSdxsdna        1447 cc-gg---c--cctcgac-----c------gggtc-----
HPdxsdna        1423 ga-gggggt--ttttgag-----cctagcggtttt-----

STdxsdna        1664 -gaaatcggcaagggtc--gcgtggtccga----gag---
CRdxsdna        1755 cgaggtgggcaagggtg--ttgtccgccgc----cag---
CJdxsdna        1444 -aaacttggtaagg-------------cac----aat---
PAdxsdna        1492 -gagatcggcaagg-----gcgtggtccgt----cggcgc
LEdxsdna        1723 -gaggttggtaaaggta--ggatattgatt----gag---
MTdxsdna        1459 -ggaggcgtggatgtgctggcggcgcccgcc---gat---
RSdxs1dna       1483 -cagatcggccgtggcc--gggtggtgagc----gag---
RSdxs2dna       1492 -gagcccggccggggcc--gcgtggtgcgc----gaa---
SPCCdxsdna      1480 -ccgattgggaaagcag--agcaactgcgc----caa---
ECdxsdna        1477 -ccaattggcaaaggca--ttgtgaagcgt----cgt---
NMdxsdna        1510 -gaaatcggcaagggca--ttatccgccgc----gaa---
HIdxsdna        1477 -cctattggtaaatcac--gtttaattcga----aaa---
SSdxsdna        1465 -g---gcggcctcgatg--tgctgcaccgc----ga----
HPdxsdna        1450 -gttttaggccaaag-c--gaattgttgaaaaaagag---
```

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```
STdxsdna      1694 ggcaagaaggtagcgatcctgtcgctcggcacg-cgcctt
CRdxsdna      1786 ggcaaggacgtgtgcctggtggcgtacggcagc-agtgtg
CJdxsdna      1463 ggcttgtaaaaaataatagtgaaatt------g-cttttt
PAdxsdna      1522 ggcggcagggtcgcactgctggtcttcggcgtg-cagttg
LEdxsdna      1753 ggggagagagtggctctattgggatatggctc--agcagt
MTdxsdna      1492 ggtttgaaccacgacgtcctgttggtggccatc-ggc---
RSdxs1dna     1513 ggcacgcgaatcgcgctcctgtccttcggcacc-cgtctg
RSdxs2dna     1522 gggacggatgtcgcgatcctctccttcggcgcg-catctg
SPCCdxsdna    1510 ggcgatgatttgctgatgttggcttacggctcg-atggtc
ECdxsdna      1507 ggcgagaaactggcgatccttaactttggtacg-ctgatg
NMdxsdna      1540 ggtgagaaaaccgcattcattgccttcggcagt-atggtc
HIdxsdna      1507 ggtcaaaaaattgcgattttaaattttggtact-ctatta
SSdxsdna      1491 --cgagcggcccgaggtgctgctggtcgccgtg-ggcgtc
HPdxsdna      1483 ggcgaaattttactcat--aggctatggtaatggcgtggg

STdxsdna      1733 gcgg--aagca-------ctaa-aggcc---------gcc
CRdxsdna      1825 aacg--aggcg-------ctgg-ccgcg---------gcg
CJdxsdna      1496 taggttatgga-------caag-gtgtg---------gca
PAdxsdna      1561 gcgg--aggcg-------atga-aggtc---------gcc
LEdxsdna      1791 gcag--aactg-------tttggatgct---------gct
MTdxsdna      1528 gcgt--tcgca-------ccga-tggcgttggcggtggcc
RSdxs1dna     1552 gccg--aggtg-------cagg-tggcc---------gcc
RSdxs2dna     1561 cacg--aggcc-------ttgc-aggcg---------gcg
SPCCdxsdna    1549 tatc--cggcc-------ctgc-agacg---------gca
ECdxsdna      1546 ccag--aagcg-------gcga-aagtc---------gcc
NMdxsdna      1579 gccc--ctgca-------ttgg-cggtc---------gcc
HIdxsdna      1546 ccat--ccgct-------ttag-agtta---------tca
SSdxsdna      1528 atgg--ca-caggtctgcctcc-agacc---------gcc
HPdxsdna      1521 gcgg--gcgca-------ttta-----g---------tcc

STdxsdna      1754 gacacgctcgaggcc--aagggcctctcgaccaccg----
CRdxsdna      1846 gacatgctggagcgc--gatggcgtgtccaccaccg----
CJdxsdna      1519 aaagcgtggcaagtcttaagagccttgcaagaaatgaata
PAdxsdna      1582 gaaagcctcgacg----------------ccacgg----
LEdxsdna      1813 attgtgctagaatcc--cgcggcttacaagtaacag----
MTdxsdna      1558 aagcggctgcacaac--caggggatcggtgtgacgg----
RSdxs1dna     1573 gaggcgctggctgcg--cgcgggatctctcccacgg----
RSdxs2dna     1582 aaacttctcgaggcc--gaggggtgagcgtgaccg----
SPCCdxsdna    1570 gaactgctgaatgag--cacggcatctcagctactg----
ECdxsdna      1567 gaatcgctgaacg----------------ccacgc----
NMdxsdna      1600 ggaaaactgaacg----------------ccaccg----
HIdxsdna      1567 gaaaaactcaatg----------------caacgg----
SSdxsdna      1555 gagctgctccgggcc--cgcggcatcggatgcacgg----
HPdxsdna      1538 aactggctttaaaag--aaaaaaacatagaatgcgc----
```

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```
STdxsdna        1788 ------------tcgcc--gacctgcgcttcgccaaaccg
CRdxsdna        1880 ------------tcatt--gacgcgcgcttctgcaagcct
CJdxsdna        1559 ataatgctaatttgatt--gatttaatttttgctaaacct
PAdxsdna        1601 ------------tcgtc--gacatgcgtttcgtcaaaccc
LEdxsdna        1847 ------------ttgca--gatgcacgtttctgcaaacca
MTdxsdna        1592 ------------tgatc--gacccgcgctgggtgttgccg
RSdxs1dna       1607 ------------ttgcg--gatgcgcgctttgcaaagccg
RSdxs2dna       1616 ------------tggcc--gacgcccgcttctcgcgcccg
SPCCdxsdna      1604 ------------tgatc--aatgcccgcttcgccaagccc
ECdxsdna        1586 ------------tggtc--gatatgcgttttgtgaaaccg
NMdxsdna        1619 ------------tcgcc--gatatgcgcttcgtcaaaccg
HIdxsdna        1586 ------------ttgtc--gatatgcgttttgtgaaaccg
SSdxsdna        1589 ------------tcgtc--gacccgcgctgggtcaagccc
HPdxsdna        1572 ------------tctcttggatctcaggtttttaaagcct

STdxsdna        1814 ctcgacgaggatctgatcc-gc-c-gcctgctcaccaccc
CRdxsdna        1906 ctggacaccaagctgatcc-gctc-ggctgc-caaggagc
CJdxsdna        1597 ttagatgaagagcttttgt-gt-gagcttgctaaaaaaag
PAdxsdna        1627 ctcgacgaagccctggtac-gc-g-aattggcgggcagcc
LEdxsdna        1873 ctggaccatgccctcataa-gg-a-gccttgcaaaatcac
MTdxsdna        1618 gtgtctgacggtgtg---c-gc-g-aactggcggtgcagc
RSdxs1dna       1633 ctcgaccgggatctgat----c-c-tgcagctcgcggccc
RSdxs2dna       1642 ctcgacacggggcacatcg-ac-c-agctcgtgcgccatc
SPCCdxsdna      1630 ttagatgaggaactgattgtgc-c-gctggcgcgccagat
ECdxsdna        1612 cttgatgaagcgttaattc-tg-g-aaatggccgccagcc
NMdxsdna        1645 atagacgaagagttgattg-tc-c-gccttgcccgaagcc
HIdxsdna        1612 attgatattgaaatgatta-at-gtgcttgcacaa-actc
SSdxsdna        1615 gtcgaccccgtgctg--------c-ccccactcgccgccg
HPdxsdna        1600 ttagatccaaatttaagcg-cg-a-tcgttgcccccttatc

STdxsdna        1851 acgaagtggcggtga---cgatcgaggaa--ggcgc---g
CRdxsdna        1943 accctgtcatgatca---ccatcgaggag--ggctc---c
CJdxsdna        1635 taaaatttggtttat---ttttagtgaaaatgttaa---a
PAdxsdna        1664 acgaactgctggtga---ccatcgaggaa--aacgccgtg
LEdxsdna        1910 atgaagtgctaatca---ctgtcgaagaa--ggatc---a
MTdxsdna        1652 acaagctgctcgtca---cgctagaggac--aacgg---g
RSdxs1dna       1667 atcacgaggcgctcattaccatcgaggag--ggcgc---c
RSdxs2dna       1679 acgcggcgctggtaa---cggtggagcag--ggggc---c
SPCCdxsdna      1668 cggcaaagtcg-tca---cctttgaggaa--ggctg---c
ECdxsdna        1649 atgaagcgctggtca---ccgtagaagaa--aacgc---c
NMdxsdna        1682 acgaccgcatcgtta---cccttgaagaa--aacgcc--g
HIdxsdna        1649 acgattatttggtca---cattggaagaa--aatgc---a
SSdxsdna        1646 agcaccggctcgtcg---ccgtcgtggag--gac------
HPdxsdna        1637 aaaagctctatgttt---ttagcgataat--tacaa---g
```

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```
STdxsdna      1883 atc--ggcggccccggt-gcgc-atgtgctgacg------
CRdxsdna      1975 gtg--ggtggcttcgct-gcgc-acgtgatgcag------
CJdxsdna      1669 att--ggcggtatagaaagttt-aattaataatt------
PAdxsdna      1699 atg--ggcggcgccggc-tcg------gcggtcggcgagt
LEdxsdna      1942 att--ggaggttttgga-tctc-atgttgttcag------
MTdxsdna      1684 gtc--aacggtggggcg-gggt-cagcggtg---------
RSdxs1dna     1702 atc--ggcggcttcggc-agcc-atgtggcgcag------
RSdxs2dna     1711 atg--ggcggcttcggc-gcct-atgtcatgcactgt---
SPCCdxsdna    1699 cta--cccggcggcttt-ggct-ccgcgattatg------
ECdxsdna      1681 att--atgggc-----g-gcgc-agg--------------
NMdxsdna      1715 aacagggcggcgcaggc-agcg-cggtgctggaa------
HIdxsdna      1681 att--caagg---tgga-gcgggatctgctgttg------
SSdxsdna      1675 aac--agccgggccgcc-gggg-tcggttcggcg------
HPdxsdna      1669 ctt--ggagg----ggt-g--------------g------

STdxsdna      1913 --ctc---gccagcgatac-cggcc--t----gatcgacg
CRdxsdna      2005 --ttc---ctcgcactgga-gggcc--t----gctggacg
CJdxsdna      1700 --ttt---tacaaaaata---------t----gat-----
PAdxsdna      1730 tcctc---gccagcga----gggcc--t------------
LEdxsdna      1972 --ttcatggccttagat----gggc--t----tcttgatg
MTdxsdna      1711 ---tc---ggccgcgctgc-ggcgc--gcggagatcgacg
RSdxs1dna     1732 --ctt---ctggccgaggc-cgggg--t----cttcgacc
RSdxs2dna     1744 --ctc---gcca---attc-cggcg--g----cttcgacg
SPCCdxsdna    1729 --gag---tcc----ttgc-aggcccat----gatc--tg
ECdxsdna      1698 ----c---agcggcgtgaa-cgaag--t----gctgatgg
NMdxsdna      1747 --gt----gttggcgaaacacggca--t----ctgcaaac
HIdxsdna      1709 ----c---ggaagtactaa-attca--t----caggaaaa
SSdxsdna      1705 --gtc---gccctggcgct-cgggg--a----cgccgatg
HPdxsdna      1682 --cta---gc--gcgattt-tagag--t----ttttga--

STdxsdna      1941 ---ccggcctc---aagc---------tgcgcaccatgcg
CRdxsdna      2033 ---gcgggctc---aagt---------tccggcccatgac
CJdxsdna      1717 ----ttgcatgtaaaagt---------tgttagctttgaa
PAdxsdna      1749 ----cgaagtc---ccgc---------tgctgcaactggg
LEdxsdna      2000 ---gcaagttg---aagt---------ggagaccaatagt
MTdxsdna      1742 tgccctgccgc---gatg---------t------cgggtt
RSdxs1dna     1760 ---gcggcttc---cggt---------atcgctcgatggt
RSdxs2dna     1769 ---ggggcctc---gcgc---------tccgggtcatgac
SPCCdxsdna    1753 ---cagg--tt---ccgg---------tgttgccgatcgg
ECdxsdna      1724 ---cccatcgt---aaaccagtacccgtgctgaacattgg
NMdxsdna      1775 ---ccg---tc---ttgc---------t------tttggg
HIdxsdna      1735 ---tcaaccgc---a-ct---------tttacaacttg-g
SSdxsdna      1733 ---tcgacgta---ccgg---------tgcgccgcttcgg
HPdxsdna      1706 ----------------------------gcgaac------
```

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```
STdxsdna      1966 cctgccggaca----tattccaggaccaggacaagcccga
CRdxsdna      2058 gctgccggacc----gctacatcgaccacggcgactaccg
CJdxsdna      1744 tatga-agaca----aatttattgaacatggaaa------
PAdxsdna      1773 cctgcccgact----actacgtcgaacacgccaagcccag
LEdxsdna      2025 tcttcctgatc----gatacattgaccatggatctcctgt
MTdxsdna      1764 gccgcaggagt----tctacgagcacgcgtctcgaagcga
RSdxs1dna     1785 gctgcccgaca----cgttcatcgaccacaacagcgccga
RSdxs2dna     1794 gctgcccgacc----gcttcatcgagcaggcgagccccga
SPCCdxsdna    1776 tgttcccgatc----tcttggtggaacatgccagccctga
ECdxsdna      1758 cctgccggact----tctt-------------tattccgc
NMdxsdna      1791 cgttgccgata----ccgtaaccggacacggcgatccgaa
HIdxsdna      1758 cttgccagattattttattccacaagcgacaca---gcaa
SSdxsdna      1758 catccccgagc----agttcctcgcgcacgccaggcgcgg
HPdxsdna      1712 -------aaaa----tattttaaagcctgttaaaagcttt

STdxsdna      2002 g------aagcagt-a--------tgacgaa--------g
CRdxsdna      2094 c------gaccagc-t--------ggccatg--------g
CJdxsdna      1773 -------aacaagt-------------gag--------g
PAdxsdna      1809 c------gagatgc-t--------cgccgaa--------t
LEdxsdna      2061 t------gatcagt-t--------ggcggaa--------g
MTdxsdna      1800 g------gtgctg------------gccgat--------c
RSdxs1dna     1821 a------gtgatgt-a--------tgccacc--------g
RSdxs2dna     1830 g------gacatgt-a--------tgccgat--------g
SPCCdxsdna    1812 tgaatctaaacagg-agttgggcctgacg----------c
ECdxsdna      1781 a------aggaactca--------ggaagaa--------a
NMdxsdna      1827 a------aaacttt-t--------agacgat--------t
HIdxsdna      1795 g------aagca-t-t--------ggcagat--------t
SSdxsdna      1794 t------gaggtgc-t--------cgccgac--------a
HPdxsdna      1741 g------aaatcat----------tgatgaatttatcatg

STdxsdna      2019 cggg-gctgaacgccgcc--------aacatcgtc-----
CRdxsdna      2111 ccgg-cctcaccagccag--------cacatcgcc-----
CJdxsdna      1784 tgga-aaaaaatctagaa--------aaagatgtc-----
PAdxsdna      1826 gcgg-cctggatgccgcg--------ggcatcg-------
LEdxsdna      2078 ctgg-cctaacaccatct--------cacattgca-----
MTdxsdna      1814 tggg-gctta---ccgac--------caggacgt------
RSdxs1dna     1838 ccgg-gctgaatgcggcc--------gacatagag-----
RSdxs2dna     1847 cggg-gctgcgggccgag--------gatatcgcg-----
SPCCdxsdna    1841 cgcg-tcagatggccgat--------cgcatcctc-----
ECdxsdna      1799 tgcgcgccgaactcggcc------------tcgat-----
NMdxsdna      1844 tggg-cttgagtgc--------------------c-----
HIdxsdna      1811 tagg-attggatacaaaa--------ggcattgaa-----
SSdxsdna      1811 tcgg-gctgaccccggtg--------gagatcgcc-----
HPdxsdna      1765 catg-g--gaacaccgctttagtggaaaaatccttaggat
```

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```
STdxsdna      2045 --gacacggtgc---tgaagg-cgctc---cgctacaacg
CRdxsdna      2137 --tccaccgcgc---tcacca-ccctggggcgcgccaagg
CJdxsdna      1810 --aatagtttgt---tgacg----------------aaag
PAdxsdna      1850 ---------------aaaagg-cagta---cg--------
LEdxsdna      2104 --gcaacagtat---ttaaca-tactt---gg-----aca
MTdxsdna      1836 --ggcccggcg-----------gatc---accggctggg
RSdxs1dna     1864 --cggaaggcgc---tggaga-cgct-------------
RSdxs2dna     1873 --gccaccgcgc---ggggcg-cgctcg--cccgggggcg
SPCCdxsdna    1867 --gaaaagtt-----tggaag-c---------cgtcaacg
ECdxsdna      1822 --gccgctggta---tggaag-c---------caaaatca
NMdxsdna      1858 --gaagcggtg-----gaacg-gcgtg---tgcg------
HIdxsdna      1837 --gaaaaaattc---tcaa-----ctt---tattgcaa--
SSdxsdna      1837 --g-ggcggatc---gg--cg-cgagc---ctgcccgtgc
HPdxsdna      1802 tagacacagagagtttgactgacgcta---ttttaaaaga

STdxsdna      2076 ---ag----gccgag-----ctggccga-cgg----gg-t
CRdxsdna      2171 ---ac----gccgccaagttctcactgt-cag----cgct
CJdxsdna      1829 ---tt----ttaaaa-----ttttatca------------
PAdxsdna      1863 -----------ccag-----cgtctcga-c----------
LEdxsdna      2130 ---aa----ccagag-----a-ggctct-aga----gg-t
MTdxsdna      1859 ---tc----gccgcg-----ctgggtac-cgg----gg-t
RSdxs1dna     1884 ----g----ggggtg-----gaggtcct-cgc----cc-g
RSdxs2dna     1905 ---cgtgatgccgct-----ccggcaga-cggcaaagc-c
SPCCdxsdna    1890 ---ga----ttggtg-----ctg--ctt-cgg----ct-t
ECdxsdna      1847 ---ag----gcctgg-----ct------------------
NMdxsdna      1881 ----c----gcgtgg-----ctgtcggatcgg----ga-t
HIdxsdna      1862 --------------------------aa-caa----gg-t
SSdxsdna      1865 ---gg----gaggaa-----ccggccga-gga----gc-a
HPdxsdna      1839 tttag----gacaag-----agagatga------------

STdxsdna      2098 gcgggcg--taa--------------------------
CRdxsdna      2199 gcaagcg--taa--------------------------
CJdxsdna      1845 ------t--taa--------------------------
PAdxsdna      1876 -cggcag--tag--------------------------
LEdxsdna      2151 catgaca--taa-------------------------
MTdxsdna      1881 gtgtgcg--tccgacgcgattccagaacatctcgactaa
RSdxs1dna     1905 ccgcgcc--tga--------------------------
RSdxs2dna     1935 gcgggcg--gtctga---------------------
SPCCdxsdna    1910 ga-----------------------------------
ECdxsdna      1857 ---ggca--taa--------------------------
NMdxsdna      1903 gcggcaaattaa-------------------------
HIdxsdna      1870 a-attta--taa--------------------------
SSdxsdna      1887 gcccgca--tga--------------------------
HPdxsdna      1853 -----------------------------------
```

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```
STdxsp          182 ----------------------------------------
AAdxsp            1 ----------------------------------------
BSdxsp            1 ----------------------------------------
CRdxsp            1 mlrgavshgpa------------------------------
CJdxsp            1 ----------------------------------------
PAdxsp            1 mpkt------------------------------------
LEdxsp            1 ----------------------------------------
MLdxsp            1 ----------------------------------------
MTdxsp            1 ----------------------------------------
RCdxsp            1 ----------------------------------------
RSdxs1p           1 ----------------------------------------
RSdxs2p           1 mtn-------------------------------------
SPCCdxsp          1 ----------------------------------------
SPdxsp            1 ----------------------------------------
TMdxsp            1 ----------------------------------------
ECdxsp            1 m---------------------------------------
NMdxsp            1 ----------------------------------------
HIdxsp            1 m---------------------------------------
PFdxsp            1 mifnyvffknfvpvvlyilliiyinlngmnnknqikteki
SSdxsp            1 ----------------------------------------
HPdxsp            1 ----------------------------------------

STdxsp          182 ----------------------------------------
AAdxsp            1 ----------------------------------------
BSdxsp            1 ----------------------------------------
CRdxsp           12 ----------------------------------------
CJdxsp            1 ----------------------------------------
PAdxsp            5 ----------------------------------------
LEdxsp            1 ----------------------------------------
MLdxsp            1 ----------------------------------------
MTdxsp            1 ----------------------------------------
RCdxsp            1 ----------------------------------------
RSdxs1p           1 ----------------------------------------
RSdxs2p           4 ----------------------------------------
SPCCdxsp          1 ----------------------------------------
SPdxsp            1 ----------------------------------------
TMdxsp            1 ----------------------------------------
ECdxsp            2 ----------------------------------------
NMdxsp            1 ----------------------------------------
HIdxsp            2 ----------------------------------------
PFdxsp           41 yikklnrlsrknslcssknkiaclfdignddnrnttygyn
SSdxsp            1 ----------------------------------------
HPdxsp            1 ----------------------------------------
```

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```
STdxsp          182 ----------------------------------------
AAdxsp            1 ----------------------------------------
BSdxsp            1 ----------------------------------------
CRdxsp           12 ----------------------------------------
CJdxsp            1 ----------------------------------------
PAdxsp            5 ----------------------------------------
LEdxsp            1 ----------------------------------------
MLdxsp            1 ----------------------------------------
MTdxsp            1 ----------------------------------------
RCdxsp            1 ----------------------------------------
RSdxs1p           1 ----------------------------------------
RSdxs2p           4 ----------------------------------------
SPCCdxsp          1 ----------------------------------------
SPdxsp            1 ----------------------------------------
TMdxsp            1 ----------------------------------------
ECdxsp            2 ----------------------------------------
NMdxsp            1 ----------------------------------------
HIdxsp            2 ----------------------------------------
PFdxsp           81 vnvknddinsllknnysnklymdkrkninnvistnkisgs
SSdxsp            1 ----------------------------------------
HPdxsp            1 ----------------------------------------

STdxsp          182 ----------------------------------------
AAdxsp            1 ----------------------------------------
BSdxsp            1 ----------------------------------------
CRdxsp           12 ----------------------------------------
CJdxsp            1 ----------------------------------------
PAdxsp            5 ----------------------------------------
LEdxsp            1 ----------------------------------------
MLdxsp            1 ----------------------------------------
MTdxsp            1 ----------------------------------------
RCdxsp            1 ----------------------------------------
RSdxs1p           1 ----------------------------------------
RSdxs2p           4 ----------------------------------------
SPCCdxsp          1 ----------------------------------------
SPdxsp            1 ----------------------------------------
TMdxsp            1 ----------------------------------------
ECdxsp            2 ----------------------------------------
NMdxsp            1 ----------------------------------------
HIdxsp            2 ----------------------------------------
PFdxsp          121 isnicsrnqkeneqkrnkqrcltqchtynmsheqdkland
SSdxsp            1 ----------------------------------------
HPdxsp            1 ----------------------------------------
```

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```
STdxsp          182 ----------------------------------------
AAdxsp            1 ----------------------------------------
BSdxsp            1 ----------------------------------------
CRdxsp           12 ----------------------------------------
CJdxsp            1 ----------------------------------------
PAdxsp            5 ----------------------------------------
LEdxsp            1 ----------------------------------------
MLdxsp            1 ----------------------------------------
MTdxsp            1 ----------------------------------------
RCdxsp            1 ----------------------------------------
RSdxs1p           1 ----------------------------------------
RSdxs2p           4 ----------------------------------------
SPCCdxsp          1 ----------------------------------------
SPdxsp            1 ----------------------------------------
TMdxsp            1 ----------------------------------------
ECdxsp            2 ----------------------------------------
NMdxsp            1 ----------------------------------------
HIdxsp            2 ----------------------------------------
PFdxsp          161 nnrnnkknfnllfinyfnlkrmknsllnkdnffyckekkl
SSdxsp            1 ----------------------------------------
HPdxsp            1 ----------------------------------------

STdxsp          182 ----------------------------------------
AAdxsp            1 ----------------------------------------
BSdxsp            1 ----------------------------------------
CRdxsp           12 ----------------------------------------
CJdxsp            1 ----------------------------------------
PAdxsp            5 ----------------------------------------
LEdxsp            1 ----------------------------------------
MLdxsp            1 ----------------------------------------
MTdxsp            1 ----------------------------------------
RCdxsp            1 ----------------------------------------
RSdxs1p           1 ----------------------------------------
RSdxs2p           4 ----------------------------------------
SPCCdxsp          1 ----------------------------------------
SPdxsp            1 ----------------------------------------
TMdxsp            1 ----------------------------------------
ECdxsp            2 ----------------------------------------
NMdxsp            1 ----------------------------------------
HIdxsp            2 ----------------------------------------
PFdxsp          201 sflhkaykkknctfqnyslkrksnrdshklfsgefddytn
SSdxsp            1 ----------------------------------------
HPdxsp            1 ----------------------------------------
```

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```
STdxsp      182 ----------------------------------------
AAdxsp        1 ----------------------------------------
BSdxsp        1 ----------------------------------------
CRdxsp       12 ----------------------------------------
CJdxsp        1 ----------------------------------------
PAdxsp        5 ----------------------------------------
LEdxsp        1 ----------------------------------------
MLdxsp        1 ----------------------------------------
MTdxsp        1 ----------------------------------------
RCdxsp        1 ----------------------------------------
RSdxs1p       1 ----------------------------------------
RSdxs2p       4 ----------------------------------------
SPCCdxsp      1 ----------------------------------------
SPdxsp        1 ----------------------------------------
TMdxsp        1 ----------------------------------------
ECdxsp        2 ----------------------------------------
NMdxsp        1 ----------------------------------------
HIdxsp        2 ----------------------------------------
PFdxsp      241 nnalyesekkeyitlnnnnknnnnknndnknndnndynnn
SSdxsp        1 ----------------------------------------
HPdxsp        1 ----------------------------------------

STdxsp      182 ----------------------------------------
AAdxsp        1 ----------------------------------------
BSdxsp        1 ----------------------------------------
CRdxsp       12 ----------------------------------------
CJdxsp        1 ----------------------------------------
PAdxsp        5 ----------------------------------------
LEdxsp        1 ----------------------------------------
MLdxsp        1 ----------------------------------------
MTdxsp        1 ----------------------------------------
RCdxsp        1 ----------------------------------------
RSdxs1p       1 ----------------------------------------
RSdxs2p       4 ----------------------------------------
SPCCdxsp      1 ----------------------------------------
SPdxsp        1 ----------------------------------------
TMdxsp        1 ----------------------------------------
ECdxsp        2 ----------------------------------------
NMdxsp        1 ----------------------------------------
HIdxsp        2 ----------------------------------------
PFdxsp      281 nscnnlgersnhydnyggdnnnpcnnnndkydigkyfkqi
SSdxsp        1 ----------------------------------------
HPdxsp        1 ----------------------------------------
```

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```
STdxsp      182 ------------------------------------m
AAdxsp        1 ------------------------------------
BSdxsp        1 ------------------------------------
CRdxsp       12 ------------------------------------v
CJdxsp        1 ------------------------------------
PAdxsp        5 ------------------------------------l
LEdxsp        1 ------------------------------------m
MLdxsp        1 ------------------------------------
MTdxsp        1 ------------------------------------
RCdxsp        1 ------------------------------------m
RSdxs1p       1 ------------------------------------m
RSdxs2p       4 ------------------------------------p
SPCCdxsp      1 ------------------------------------
SPdxsp        1 ------------------------------------
TMdxsp        1 ------------------------------------
ECdxsp        2 ------------------------------------s
NMdxsp        1 ------------------------------------
HIdxsp        2 ------------------------------------t
PFdxsp      321 ntfinideyktiygdeiykeiyelyvernipeyyerkyfs
SSdxsp        1 ------------------------------------
HPdxsp        1 ------------------------------------m

STdxsp      185 a--------------------------------dl----
AAdxsp        1 --------------------------------ml----
BSdxsp        1 ------------------------------------
CRdxsp       13 a--------------------------------draaag
CJdxsp        1 ----------------------------------m----
PAdxsp        6 h----------------------------------ei----
LEdxsp        2 alcayafpgilnrtgvvsdsskatplfsgwihgtdlqflf
MLdxsp        1 ------------------------------------
MTdxsp        1 ------------------------------------
RCdxsp        2 s--------------------------------at----
RSdxs1p       2 t--------------------------------dr----
RSdxs2p       5 t--------------------------------pr----
SPCCdxsp      1 ------------------------------------
SPdxsp        1 ------------------------------------
TMdxsp        1 ------------------------------------
ECdxsp        3 f--------------------------------di----
NMdxsp        1 ----------------------------------m----
HIdxsp        3 n--------------------------------nm----
PFdxsp      361 e--------------------------------di----
SSdxsp        1 ------------------------------------
HPdxsp        2 i--------------------------------lq----
```

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```
STdxsp          194 ----------------------------------------
AAdxsp            3 ----------------------------------------
BSdxsp            1 ----------------------------------------
CRdxsp           20 parcaapvargvrsaaptrqrraeasvnapragpagsysg
CJdxsp            2 ----------------------------------------
PAdxsp            9 ----------------------------------------
LEdxsp           42 qhklthevkkrsrvvqaslsesgeyytqr-----------
MLdxsp            1 ----------------------------------------
MTdxsp            1 ----------------------------------------
RCdxsp            5 ----------------------------------------
RSdxs1p           5 ----------------------------------------
RSdxs2p           8 ----------------------------------------
SPCCdxsp          1 ----------------------------------------
SPdxsp            1 ----------------------------------------
TMdxsp            1 ----------------------------------------
ECdxsp            6 ----------------------------------------
NMdxsp            2 ----------------------------------------
HIdxsp            6 ----------------------------------------
PFdxsp          364 ----------------------------------------
SSdxsp            1 ----------------------------------------
HPdxsp            5 ----------------------------------------

STdxsp          194 -----------------p-k----t---------------
AAdxsp            3 -----------------e-k----y---------------
BSdxsp            1 ----------------------m---------------
CRdxsp           60 ewdklsveeidewrdvgp-k----t---------------
CJdxsp            2 -----------------s-k--------------------
PAdxsp            9 -----------------p-rerpat---------------
LEdxsp           71 -----------------p-p----t---------------
MLdxsp            1 ----------------------------------------
MTdxsp            1 ----------------------------------------
RCdxsp            5 -----------------psr----t---------------
RSdxs1p           5 -----------------p-c----t---------------
RSdxs2p           8 -----------------p-e----t---------------
SPCCdxsp          1 ----------------------------------------
SPdxsp            1 ----------------------------------------
TMdxsp            1 ----------------------------------------
ECdxsp            6 -----------------a-k----y---------------
NMdxsp            2 -----------------n-p----s---------------
HIdxsp            6 -----------------n-n----y---------------
PFdxsp          364 -----------------k-k----svlfdidkyndvefek
SSdxsp            1 ----------------------m---------------
HPdxsp            5 -----------------n-k----t---------------
```

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```
STdxsp      203 ------------------------------------plld
AAdxsp        6 ------------------------------------eilk
BSdxsp        2 ------------------------------------dll-
CRdxsp       80 ------------------------------------plld
CJdxsp        4 ------------------------------------k
PAdxsp       16 ------------------------------------plld
LEdxsp       74 ------------------------------------pild
MLdxsp        1 ------------------------------------mle
MTdxsp        1 ------------------------------------mlq
RCdxsp        9 ------------------------------------phld
RSdxs1p       8 ------------------------------------ptld
RSdxs2p      11 ------------------------------------plld
SPCCdxsp      1 ------------------------------------mhls
SPdxsp        1 ------------------------------------mhis
TMdxsp        1 ------------------------------------mlld
ECdxsp        9 ------------------------------------ptla
NMdxsp        5 ------------------------------------plld
HIdxsp        9 ------------------------------------plls
PFdxsp      382 aikeefinngvyinnidntyykkenilimkkilhyfpllk
SSdxsp        2 ------------------------------------tile
HPdxsp        8 ------------------------------------fdln

STdxsp      215 tvdtpqdlrklapaqlrqladelraetisavgstgghlgs
AAdxsp       10 dykgpfdiknydyetlqklaqevrdyiinvtskngghvgp
BSdxsp        5 siqdpsflknmsideleklsdeirqflitslsasgghigp
CRdxsp       84 tvnypvhlknfnneqlkqlckelrsdivhtvsrtgghlss
CJdxsp        5 fahtqeeleklslkelenlaasmrekiiqvvskngghlss
PAdxsp       20 rasspaelrrlgeadletladelrqyllytvgqtgghfga
LEdxsp       78 tvnypihmknlslkelkqladelrsdtifnvsktgghlgs
MLdxsp        4 qirrpadlqhlsqqqlrdlaaeirellvhkvaatgghlgp
MTdxsp        4 qirgpadlqhlsqaqlrelaaeireflihkvaatgghlgp
RCdxsp       13 rvtgpadlkamsiadltalasevrreivevvsqtgghlgs
RSdxs1p      12 rvtlpvdikgltdrelrsladelraetisavsvtgghlga
RSdxs2p      15 rvccpadmkalsdaelerladevrsevisvvaetgghlgs
SPCCdxsp      5 eithpnqlhglsvaqleqighqirekhlqtvaatgghlgp
SPdxsp        5 elthpnelkglsireleevsrqirekhlqtvatsgghlgp
TMdxsp        5 ------eikrmsydelkrlaedirkritevvlkngghlas
ECdxsp       13 lvdstqelrllpkeslpklcdelrrylldsvsrssghfas
NMdxsp        9 lidspqdlrrldkkqlprlagelrtfllesvgqtgghfas
HIdxsp       13 linspedlrllnkdqlpqlcqelrayllesvsqtsghlas
PFdxsp      422 linnpsdlkklkkqylpllahelkiflffivnitgghfss
SSdxsp        6 nirqprdlkalpeeqlhelseeirqflvhavtrtgghlgp
HPdxsp       12 ----pndi-----aglelvcqtlrnrilevvsangghlss
```

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```
STdxsp      335 glgvveltvaihyvfntpddrliwdvghqcyphkiltgrr
AAdxsp       50 slgvveltiallrvfnppedvivwdighqgypwkiltdrk
BSdxsp       45 nlgvveltvalhkefnspkdkflwdvghqsyvhkllgrg
CRdxsp      124 slgvveltvamhyvfntpedkiiwdvghqayghkiltgrr
CJdxsp       45 nlgavelsiamhlvfdakkdpfifdvshqsythkllsgke
PAdxsp       60 glgvveltialhyvfdtpddrlvwdvghqayphkilterr
LEdxsp       118 slgvveltvalhyvfnapqdrilwdvghqsyphkiltgrr
MLdxsp       44 nlgvveltlalhrvfdsphdpiifdtghqayvhkmltgrc
MTdxsp       44 nlgvveltlalhrvfdsphdpiifdtghqayvhkmltgrs
RCdxsp       53 slgvveltvalhavfnspgdkliwdvghqcyphkiltgrr
RSdxs1p      52 glgvveltvalhaifdaprdkiiwdvghqcyphkiltgrr
RSdxs2p      55 slgvveltvalhavfntptdklvwdvghqcyphkiltgrr
SPCCdxsp     45 glgvveltlalyqtldldrdkvvwdvghqayphklltgry
SPdxsp       45 glgvveltvalystldldkdrviwdvghqayphkmltgry
TMdxsp       39 nlgtieltlalyrvfdpredaiiwdtghqaythkiltgrd
ECdxsp       53 glgtveltvalhyvyntpfdqliwdvghqayphkiltgrr
NMdxsp       49 nlgaveltvalhyvyntpedklvwdvghqsyphkiltgrk
HIdxsp       53 glgtveltvalhyvyktpfdqliwdvghqayphkiltgrr
PFdxsp       462 vlssleiqllllyifnqpydnviydighqayvhkiltgrk
SSdxsp       46 nlgvveltialhrvfespvdrilwdtghqsyvhkllgrq
HPdxsp       43 slgavelivgmhalfdcqknpfifdtshqayahkllgrf

STdxsp      455 drirtirqggglsgftkrseseydpfgaahsstsisaalg
AAdxsp       90 eqfptlrqykgisgflrreesiydafgaghsstsisaalg
BSdxsp       85 kefatlrqykglcgfpkrsesehdvwetghsstslsgamg
CRdxsp      164 kgmatirqtnglsgftkrdeseydpfgaghsstsisaalg
CJdxsp       85 eifdtlrqinglsgytkpsegdy--fvaghsstsislavg
PAdxsp      100 elmgtlrqknglaafprraeseydtfgvghsstsisaalg
LEdxsp      158 dkmstlrqtdglagftkrseseydcfgtghssttisaglg
MLdxsp       84 qdfdslrkkaglsgypsraesehdwvesshastalsyadg
MTdxsp       84 qdfatlrkkgglsgypsraesehdwvesshasaalsyadg
RCdxsp       93 srmltlrqaggisgfpkrsesphdafgaghsstsisaalg
RSdxs1p      92 drirtlrqggglsgftkrsespydcfgaghsstsisaavg
RSdxs2p      95 eqmrtlrqkgglsgftkrsesaydpfgaahsstsisaalg
SPCCdxsp     85 hnfhtlrqkdgiagypkrtenrfdhfgaghastsisaglg
SPdxsp       85 hdfhtlrqkdgvagylkrsesrfdhfgaghastsisaglg
TMdxsp       79 dlfhtirtfgglsgfvtrrespldwfgtghagtsiaaglg
ECdxsp       93 dkigtirqkgglhpfpwrgeseydvlsvghsstsisagig
NMdxsp       89 nqmhtmrqygglagfpkrceseydafgvghsstsigaalg
HIdxsp       93 eqmstirqkdgihpfpwreesefdvlsvghsstsisaglg
PFdxsp       502 llflslrnkkgisgflnifesiydkfgaghsstslsaiqg
SSdxsp       86 d-fsklrgkgglsgypsreesehdvienshastalgwadg
HPdxsp       83 esfstlrqfkglsgftkpsesaydyfiaghsstsvsigvg
```

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```
STdxsp          575 fa-----------------------------------
AAdxsp          130 fr-----------------------------------
BSdxsp          125 ma-----------------------------------
CRdxsp          204 ma-----------------------------------
CJdxsp          123 ac-----------------------------------
PAdxsp          140 ma-----------------------------------
LEdxsp          198 ma-----------------------------------
MLdxsp          124 la-----------------------------------
MTdxsp          124 la-----------------------------------
RCdxsp          133 fa-----------------------------------
RSdxs1p         132 fa-----------------------------------
RSdxs2p         135 fa-----------------------------------
SPCCdxsp        125 ma-----------------------------------
SPdxsp          125 ma-----------------------------------
TMdxsp          119 fe-----------------------------------
ECdxsp          133 ia-----------------------------------
NMdxsp          129 ma-----------------------------------
HIdxsp          133 ia-----------------------------------
PFdxsp          542 yyeaewqvknkekygngdieisdnanvtnnerifqkgihn
SSdxsp          125 la-----------------------------------
HPdxsp          123 va-----------------------------------

STdxsp          581 ---iankln--------eapgk-a----------------
AAdxsp          132 ---igkdlkg-------ekedy-v----------------
BSdxsp          127 ---aardik--------gtdey-i----------------
CRdxsp          206 ---vgrdvk--------gkkns-v----------------
CJdxsp          125 ---kaialk--------gekri-p----------------
PAdxsp          142 ---iaarlq--------gkerk-s----------------
LEdxsp          200 ---vgrdlk--------grnnn-v----------------
MLdxsp          126 ---kafela--------gnrnrhv----------------
MTdxsp          126 ---kafelt--------ghrnrhv----------------
RCdxsp          135 ---vgrelg--------qpvgd-t----------------
RSdxs1p         134 ---aaremg--------gdtgd-a----------------
RSdxs2p         137 ---mgrelg--------qpvgd-t----------------
SPCCdxsp        127 ---lardaq--------gedyr-c----------------
SPdxsp          127 ---lardak--------gedfk-v----------------
TMdxsp          121 ---kafell--------gekrh-v----------------
ECdxsp          135 ---vaaeke--------gknrr-t----------------
NMdxsp          131 ---aadkql--------gsdrr-s----------------
HIdxsp          135 ---vaaere--------nagrk-t----------------
PFdxsp          582 dnninnninnnnyinpsdvvgr-entnvpnvrndnhnvdk
SSdxsp          127 ---karrvq--------gekgh-v----------------
HPdxsp          125 ---kafclk--------qalgm-p----------------
```

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```
STdxsp      617 --iavigdgamsagmayeamnna-eaagnr-lvvilndnd
AAdxsp      145 --iavigdgaltagmayealnnaghirpdr-fivilndne
BSdxsp      139 --ipiigdgaltggmalealnhi-gdekkd-mivilndne
CRdxsp      218 --iavigdgaitggmayeamnha-gfldkn-mivilndnq
CJdxsp      137 --valigdgalsagmayealnel-gdskfp-cvillndne
PAdxsp      154 --vavigdgaltagmafealnha-sevdad-mlvilndnd
LEdxsp      212 --iavigdgamtagqayeamnna-gyldsd-mivilndnr
MLdxsp      139 --vavvgdgaltggmcwealnni-aatprp-vvivvndng
MTdxsp      139 --vavvgdgaltggmcwealnni-aasrrp-viivvndng
RCdxsp      147 --iaiigdgsitagmayealnha-ghlksr-mfvilndnd
RSdxs1p     146 --vavigdgsmsagmafealnhg-ghlknr-vivilndne
RSdxs2p     149 --iavigdgsitagmayealnha-ghlnkr-lfvilndnd
SPCCdxsp    139 --vavigdgsltggmaleainhaghlpktr-llvvlndnd
SPdxsp      139 --vsiigdgaltggmaleainhaghlphtr-lmvilndne
TMdxsp      133 --vvvigdgaltsgmalealnql-knlnsk-mkiilndng
ECdxsp      147 --vcvigdgaitagmafeamnha-gdirpd-mlvilndne
NMdxsp      143 --vaiigdgamtagqafealnca-gdmdvd-llvvlndne
HIdxsp      147 --vcvigdgaitagmafealnha-galhtd-mlvilndne
PFdxsp      621 vhiaiigdggltggmalealnyi-sflnsk-iliiyndng
SSdxsp      139 --vaviggraltggmawealnni-aaakdqpliivvndne
HPdxsp      137 --iallgdgsisagifyealnel-gdrkyp-mimilndne

STdxsp      725 msiap----------pvgglsayl--arlissseyl--gl
AAdxsp      182 msisp----------nvgaistyl--nriisghfvq--et
BSdxsp      175 msiap----------nvgaihsml--grlrtagkyq--wv
CRdxsp      254 qvslptqynnknqd-pvgalssal--arlqanrplr--el
CJdxsp      173 msisk----------pigaiskyl--sqamatqfyq--sf
PAdxsp      190 msish----------nvgglsnyl--akilssrtys--sm
LEdxsp      248 qvslptatldgpva-pvgalssal--srlqsnrplr--el
MLdxsp      175 rsyap----------tiggvadhl--atlrlqpaye--rl
MTdxsp      175 rsyap----------tiggvadhl--atlrlqpay-----
RCdxsp      183 msiap----------pvgalqhyl--ntiarqapfa--al
RSdxs1p     182 msiap----------pvgalssyl--srlyagapfq--df
RSdxs2p     185 msiap----------pvgalaryl--vnlsskapfa--tl
SPCCdxsp    176 msisp----------nvgalsryl--nk-irvsepm--ql
SPdxsp      176 msisp----------nvgaisrylnkvrlsspmqfltdnl
TMdxsp      169 msisp----------nvgglayhl--sklrtspiyl--kg
ECdxsp      183 msise----------nvgalnnhl--aqllsgklys--sl
NMdxsp      179 msisp----------nvgalpkyl--asnvvrdmh---gl
HIdxsp      183 msise----------nvgalnnhl--arifsgslys--tl
PFdxsp      659 qvslptnavsisgnrpigsisdhl--hyfvsnie------
SSdxsp      176 rsyap----------tigglanhl--atlrttdgye--kv
HPdxsp      173 msist----------pigalskal--sqlmkgpfyq--sf
```

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```
STdxsp      803 relakrf---trk--lsr------rltaa---a-gkaeef
AAdxsp      208 rqkiknf---lqh--fge------tplri---m-klteef
BSdxsp      201 kdeleyl---fkk--ipavgg---klaat---a-ervkds
CRdxsp      289 reiakgv---tkq--lpd------vvqka---t-akidey
CJdxsp      199 kkriakm---ldi--lpd------satym---a-krfees
PAdxsp      216 regsk-------k--vls------rlpgaweia-rrteey
LEdxsp      283 revakgv---tkq--igg------pmhel---a-akvdey
MLdxsp      201 lekg-------rd--alh------slpli---g-qiayrf
MTdxsp      198 -eqalet---grd--lvr------avplv---g-glwfrf
RCdxsp      209 kaaaegi---emh--lpg------pvrdg---a-rrarqm
RSdxs1p     208 kaaakga---lgl--lpe------pfqeg---a-rrakem
RSdxs2p     211 raaadgl---eas--lpg------plrdg---a-rrarql
SPCCdxsp    201 --ltdgl---tqg--mqqipfvggaitqg---f-epvkeg
SPdxsp      206 eeqikhl---pf---vgd------sltpe---m-ervkeg
TMdxsp      195 kkvlkkv---lekteigf------eveee---m-kylrds
ECdxsp      209 reggkkv---fsg--vp-------pikel---l-krteeh
NMdxsp      204 lstvkaq---tgk--vld------kipgamefa-qkvehk
HIdxsp      209 rdgskki---ldk--vp-------piknf---m-kkteeh
PFdxsp      691 ---anag---dnk--lsk------n---------------
SSdxsp      202 lawgkdvllrtpi--vgh------plyea---lhgakkgf
HPdxsp      199 rskvkki---lst--lpe------svnyl---a-srfees

STdxsp      878 argm--atg-------g-------tlfeelgfyyvgpidg
AAdxsp      233 lkgl--isp-------g-------vifeelgfnyigpidg
BSdxsp      229 lkym--lvs-------g-------mffeelgftylgpvdg
CRdxsp      314 argmisgtg-------s-------tlfeelglyyigpvdg
CJdxsp      224 fk-l--itp-------g-------llfeelgleyigpidg
PAdxsp      240 akgm--lvp-------g-------tlfeelgwnyigpidg
LEdxsp      308 argmisgsg-------s-------tlfeelglyyigpvdg
MLdxsp      222 mhsv--kagikdslspq-------llftdlglkyvgpvdg
MTdxsp      222 lhsv--kagikdslspq-------llftdlglkyvgpvdg
RCdxsp      234 vtam--pgg-------a-------tlfeelgfdyigpvdg
RSdxs1p     233 lksv--tvg-------g-------tlfeelgfsyvgpidg
RSdxs2p     236 vtgm--pgg-------g-------tlfeelgftyvgpidg
SPCCdxsp    230 mkrl--syski-----g-------avfeelgftymgpvdg
SPdxsp      230 mkrl--vvpkv-----g-------avieelgfkyfgpidg
TMdxsp      222 lkgm--iqg-------t-------nffeslglkyfgpfdg
ECdxsp      233 ikgm--vvp-------g-------tlfeelgfnyigpvdg
NMdxsp      232 iktl--aee-------aehakqslslfenfgfrytgpvdg
HIdxsp      233 mkgvmfspe-------s-------tlfeelgfnyigpvdg
PFdxsp      702 ------ake-------n-------nifenlnydyigvvng
SSdxsp      231 kdaf--apq-------g--------mfedlglkyvgpidg
HPdxsp      224 fk-l--itp-------g-------vffeelginyigping
```

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```
STdxsp          950 hnlehlipvlenvrdse-q-gpilihvvtkkgkgyapaea
AAdxsp          257 hdikaledtlnnvkdi--k-gpvllhvytkkgkgykpaee
BSdxsp          253 hsyhelienlqyakkt--k-gpvllhvitkkgkgykpaet
CRdxsp          340 hnlddliavlsevrsae-tvgpvlvhvvtekgrgylpaet
CJdxsp          247 hnlgeiisalkqak-am-q-kpcvihaqtikgkgyalaeg
PAdxsp          264 hdlptlvatlrnmrdm--k-gpqflhvvtkkgkgfapael
LEdxsp          334 hniddliailkevrstk-ttgpvlihvvtekgrgypyaer
MLdxsp          253 hd-ehavevalrkargf-g-gpvivhvvtrkgmgyppaea
MTdxsp          253 hd-eravevalrsarrf-g-apvivhvvtrkgmgyppaea
RCdxsp          258 hdmaelvetlrvtrara-s-gpvlihvcttkgkgyapaeg
RSdxs1p         257 hdldqllpvlrtvkqra-h-apvlihvitkkgrgyapaea
RSdxs2p         260 hdmeallqtlraarart-t-gpvlihvvtkkgkgyapaen
SPCCdxsp        256 hnleeliatfreah-kh-t-gpvlvhvattkgkgypyaee
SPdxsp          256 hslqelidtfkqa-ekv-p-gpvfvhvsttkgkgydlaek
TMdxsp          246 hniellekvfkrirdyd-y-ssv-vhvvtkkgkgftaaee
ECdxsp          257 hdvlglittlknmrdl--k-gpqflhimtkkgrgyepaek
NMdxsp          263 hnvenlvdvledlr-gr-k-gpqllhvitkkgngyklaen
HIdxsp          259 hnidelvatltnmrnl--k-gpqflhiktkkgkgyapaek
PFdxsp          722 nnteelfkvlnnikenklk-ratvlhvrtkksndfinsks
SSdxsp          254 hdigavesalrrak-rf-h-gpvlvhcltvkgrgyepala
HPdxsp          247 hdlsaiietlklakelk-e--pvlihaqtlkgkgykiaeg

STdxsp         1064 -aadkyhgvqk-----fd--vitg-aqaka-----pp---
AAdxsp          294 -npvkwhgvap-----yk--vesg-eiik------ks---
BSdxsp          290 dtigtwhgtgp-----yk--intg-dfvkp-----ka---
CRdxsp          379 -aqdkmhgvvk-----fd--prtg-kqvqa-----kt---
CJdxsp          284 -khakwhgvga-----fd--idsg-esvkk-----sd---
PAdxsp          301 -dpigyhaitk-----le--apgs-apkkt----------
LEdxsp          373 -aadkyhgvak-----fd--patg-kqfka-----sa---
MLdxsp          290 dqaeqmhtcgv-----md--pttg-qptki----------
MTdxsp          290 dqaeqmhstvp-----id--patg-qatkv----------
RCdxsp          296 -aedklhgvsk-----fd--ietg-kqkks-----ip---
RSdxs1p         295 -ardrghatnk-----fn--vltg-aqvkp-----vs---
RSdxs2p         298 -apdkyhgvnk-----fd--pvtg-eqkks-----va---
SPCCdxsp        293 -dqvgyhaqnp-----fd--latgkakpas-----kp---
SPdxsp          293 -dqvgyhaqsp-----fn--lstgkaypss-----kp---
TMdxsp          283 -nptkyh-------------------sas-----ps---
ECdxsp          294 -dpitfhavpk-----fd--pssg-clpks-----sg---
NMdxsp          300 -dpvkyhavan-----lp--kesa-aqmpsekepkpa---
HIdxsp          296 -dpigfhgvpk-----fd--pisg-elpk------nn---
PFdxsp          761 -pisilhsikkneifpfdttilng-nihke-----nkiee
SSdxsp          291 heedhfhtvgv-----md--plt--cepls-----pt---
HPdxsp          284 -ryekwhgvgp-----fd--ldtg-lskks-----ks---
```

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```
STdxsp      1133 ----------------------------------gpp---ay
AAdxsp       316 ----------------------------------spp---tw
BSdxsp       314 ----------------------------------aap---sw
CRdxsp       402 ----------------------------------kam---sy
CJdxsp       307 ----------------------------------tkk---sa
PAdxsp       322 ----------------------------------ggp---ky
LEdxsp       396 ----------------------------------ktq---sy
MLdxsp       312 ----------------------------------aap---dw
MTdxsp       312 ----------------------------------agp---gw
RCdxsp       319 ----------------------------------nap---ny
RSdxs1p      318 ----------------------------------nap---sy
RSdxs2p      321 ----------------------------------nap---ny
SPCCdxsp     317 ----------------------------------kpp---sy
SPdxsp       317 ----------------------------------kpp---sy
TMdxsp       294 ----------------------------------gkpkmlsy
ECdxsp       317 ----------------------------------glp---sy
NMdxsp       328 ----------------------------------akp---ty
HIdxsp       318 ----------------------------------skp---ty
PFdxsp       794 eknvssstkydvnnknnknndnseiikyedmfske---tf
SSdxsp       314 ----------------------------------dgp---sw
HPdxsp       307 ----------------------------------ail---sp

STdxsp      1148 tkvfadallaeaerdasvcaitaampsgtgldkfqatfpd
AAdxsp       321 tsvfgkalvelaerdekivaitpamregsglvefakrfpd
BSdxsp       319 sglvsgtvqrmaredgrivaitpampvgsklegfakefpd
CRdxsp       407 tnyfadaltaeaerdsrivavhaamaggtglyrfekkfpd
CJdxsp       312 teifsknlidlaskyenivgvtaampsgtgldkliekypn
PAdxsp       327 ssvfgqwlcdmaaqdarllgitpamkegsdlvafserype
LEdxsp       401 ttyfaealiaeaeadkdivaihaamgggtgmnlfhrrfpt
MLdxsp       317 taifsdaligyamkrrdivaitaampgptgltafgqcfpd
MTdxsp       317 tatfsdaligyaqkrrdivaitaampgptgltafgqrfpd
RCdxsp       324 tavfgerlteeaardqaivavtaamptgtgldimqkrfpr
RSdxs1p      323 tkvfaqslikeaevdericavtaampdgtglnlfgerfpk
RSdxs2p      326 tkvfgstlteeaardprivaitaampsgtgvdimqkrfpn
SPCCdxsp     322 skvfgqtlttlaksdrrivgitaamatgtgldilqkalpk
SPdxsp       322 skvfahtlttlakenpnivgitaamatgtgldklqaklpk
TMdxsp       302 sellghtlsrvaredkkivaitaamadgtglsifqkehpd
ECdxsp       322 skifgdwlcetaakdnklmaitpamregsgmvefsrkfpd
NMdxsp       333 tqvfgkwlcdraaadsrlvaitpamregsglvefeqrfpd
HIdxsp       323 skifgdwlcemaekdakiigitpamregsgmvefsqrfpk
PFdxsp       831 tdiytnemlkylkkdrniiflspamlggsglvkiserypn
SSdxsp       319 tsvfgdeivrigaeredivaitaamlhpvglarfadrfpd
HPdxsp       312 teaysntllelakkdekivgvtaampsgtgldklidaypl
```

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```
STdxsp      1268 rtfdvaiaeqhavtfaaglaa-qgmrpfcaiystflqray
AAdxsp       361 rffdvgiaeqhactfaaglaa-eglrpvaayystflqray
BSdxsp       359 rmfdvgiaeqhaatmaaamam-qgmkpflaiystflqray
CRdxsp       447 rtfdvgiaeqhavtfaaglac-eglvpfctiystfmqrgy
CJdxsp       352 rfwdvaiaeqhavtsmaamak-egfkpfiaiystflqray
PAdxsp       367 ryfdvaiaeqhavtlaagmac-egmkpvvaiystflqray
LEdxsp       441 rcfdvgiaeqhavtfaaglac-egikpfcaiyssfmqray
MLdxsp       357 rlfdvgiaeqhamtsaaglam-grmhpvvaiystflnraf
MTdxsp       357 rlfdvgiaeqhamtsaaglam-gglhpvvaiystflnraf
RCdxsp       364 rvfdvgiaeqhavtfaagmaa-aglkpflalyssfvqrgy
RSdxs1p      363 rtfdvgiaeqhavtfsaalaa-ggmrpfcaiystflqrgy
RSdxs2p      366 rvfdvgiaeqhavtfaaglag-agmkpfcaiyssflqrgy
SPCCdxsp     362 qyidvgiaeqhavvlaagmac-dgmrpvvaiystflqraf
SPdxsp       362 qyvdvgiaeqhavtlaagmac-egirpvvaiystflqrgy
TMdxsp       342 rffdlgiteqtcvtfgaalgl-hgmkpvvaiystflqray
ECdxsp       362 ryfdvaiaeqhavtfaaglai-ggykpivaiystflqray
NMdxsp       373 ryfdvgiaeqhavtfagglac-egmkpvvaiystflqray
HIdxsp       363 qyfdvaiaeqhavtfatglai-ggykpvvaiystflqray
PFdxsp       871 nvydvgiaeqhsvtfaaamamnkklkiqlciystflqray
SSdxsp       359 rvwdvgiaeqhaavsaaglat-gglhpvvavyatflnraf
HPdxsp       352 rffdvaiaeqhaltsssamak-egfkpfvsiystflqray

STdxsp      1385 dqvvhdvaiqnlpvrfaidraglvgadgathagsfdvtyl
AAdxsp       400 dqvihdvalqnlpvtfaidraglvgddgpthhgvfdlsyl
BSdxsp       398 dqvvhdicrqnanvfigidraglvgadgethqgvfdiafm
CRdxsp       486 dqivhdvslqklpvrfamdraglvgadgsthcgafdvtfm
CJdxsp       391 dqvihdcaimnlnvvfamdragivgedgethqgvfdlsfl
PAdxsp       406 dqlihdvavqhldvlfaidraglvgedgpthagsfdisyl
LEdxsp       480 dqvvhdvdlqklpvrfamdraglvgadgpthcgafdvtym
MLdxsp       396 dqimmdvalhklpvtmvidragitgsdgpshngmwdlsml
MTdxsp       396 dqimmdvalhklpvtmvldragitgsdgashngmwdlsml
RCdxsp       403 dqlvhdvalqnlpvrlmidraglvgqdgathagafdvsml
RSdxs1p      402 dqivhdvaiqrlpvrfaidraglvgadgathagsfdvafl
RSdxs2p      405 dqiahdvalqnlpvrfvidraglvgadgathagafdvgfi
SPCCdxsp     401 dqvihdvciqklpvffcldragivgadgpthqgmydiayl
SPdxsp       401 dqiihdvciqklpvffcldragivgadgpthqgmydiayl
TMdxsp       381 dqiihdvalqnapvlfaidrsgvvgedgpthhglfdinyl
ECdxsp       401 dqvlhdvaiqklpvlfaidragivgadgqthqgafdlsyl
NMdxsp       412 dqlvhdialqnlpvlfavdragivgadgpthaglydlsfl
HIdxsp       402 dqlihdvaiqnlpvlfaidragivgadgathqgafdisfm
PFdxsp       911 dqiihdlnlqniplkviigrsglvgedgathqgiydlsyl
SSdxsp       398 dqllmdvalhrcgvtfvldragvtgvdgashngmwdmsvl
HPdxsp       391 dsivhdacisslpiklaidragivgedgethqglldvsyl
```

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```
STdxsp      1505 aslpnfvvmaaadevelvhmthtaamhdsg-pialryprg
AAdxsp       440 rcvpnmvvcapkdeqelrdllytg-iysgk-pfalryprg
BSdxsp       438 rhipnmvlmmpkdenegqhmvhtalsydeg-piamrfprg
CRdxsp       526 aslphmitmapsneaelinmvatcaaidda-pscfrfprg
CJdxsp       431 aplpnftllaprdeqmmqnimeyaylh-qg-pialryprg
PAdxsp       446 rcipgmlvmtpsdedelrkllttgylfd-g-paavryprg
LEdxsp       520 aclpnmvvmapsdeaelfhmvataaaiddr-pscfryprg
MLdxsp       436 givpgmrvaaprdairlreelgealdvddg-ptairfpkg
MTdxsp       436 givpgirvaaprdatrlreelgealdvddg-ptalrfpkg
RCdxsp       443 anlpnftvmaaadeaelchmvvtaaahdsg-pialryprg
RSdxs1p      442 snlpgivvmaaadeaelvhmvataaahdeg-piafryprg
RSdxs2p      445 tslpnmtvmaaadeaelihmiatavafgeg-piafrfprg
SPCCdxsp     441 rlipnmvlmapkdeaelqrmlvtgieyd-g-piamrfprg
SPdxsp       441 rcipnlvlmapkdeaelqqmlvtgvnytgg-aiamryprg
TMdxsp       421 lpvpnmkiispsspeefvnslytvlkhldg-pvairypke
ECdxsp       441 rcipemvimtpsdenecrqmlytgyhyndg-psavryprg
NMdxsp       452 rcipnmivaapsdenecrlllstcyqada--paavryprg
HIdxsp       442 rcipnmiimtpsdenecrqmlytg--yqcgkpaavryprg
PFdxsp       951 gtlnnayiispsnqvdlkralrfayldkdh-svyiriprm
SSdxsp       438 qvvpglriaaprdadhvraqlreavavdda-ptlirfpk-
HPdxsp       431 rsipnmvifaprdnetlknavrfanehdss-pcafryprg

STdxsp      1622 n---------gvglalpk---------vp-erle------
AAdxsp       478 a---------aygvpteg---------f--kkie------
BSdxsp       477 n---------glgvkmde---------ql-ktip------
CRdxsp       565 n---------glgldlaaygiskdlkgvp---le------
CJdxsp       469 s---------fi-ldkef---------np-ceik------
PAdxsp       484 s---------gpnhpidp---------dl-qpve------
LEdxsp       559 n---------gigvelpagnkg-----ip---le------
MLdxsp       475 d----------vcedipa---------lk-rrsg------
MTdxsp       475 d----------vgedisa---------le-rrgg------
RCdxsp       482 e---------grgvempe---------rg-evle------
RSdxs1p      481 d---------gvgvevpv---------kg-vplq------
RSdxs2p      484 e---------gvgvempe---------rg-tvle------
SPCCdxsp     479 n---------gigvplpe---------egweslp------
SPdxsp       480 n---------gigvplme---------egweple------
TMdxsp       460 s---------fygevesl---------le-nmke------
ECdxsp       480 n---------avgveltp---------l--eklp------
NMdxsp       490 t---------gtgvpvsd---------gm-etve------
HIdxsp       480 n---------avgvkltp---------l--emlp------
PFdxsp       990 nilsdkymkgylnihmkn---------es-knidvnvdin
SSdxsp       476 e---------svg---pr---------ip-aldr------
HPdxsp       470 s------------falkegvf------ep-sgfv------
```

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```
STdxsp      1667 --------------------igkg-r-vvr----------
AAdxsp       492 --------------------igtw-e-ell----------
BSdxsp       492 --------------------igtw-e-vlr----------
CRdxsp       587 --------------------vgkg-v-vrr----------
CJdxsp       483 --------------------lgka-qwlvk----------
PAdxsp       499 --------------------igkg-v-vrr----------
LEdxsp       576 --------------------vgkg-r-ili----------
MLdxsp       489 --------------------vdvl-a-vpa----------
MTdxsp       489 --------------------vdvl-a-apa----------
RCdxsp       497 --------------------igkg-r-vmt----------
RSdxs1p      496 --------------------igrg-r-vvs----------
RSdxs2p      499 --------------------pgrg-r-vvr----------
SPCCdxsp     495 --------------------igka-e-qlr----------
SPdxsp       496 --------------------igka-e-ilr----------
TMdxsp       475 --------------------idlgwk-ilk----------
ECdxsp       494 --------------------igkg-i-vkr----------
NMdxsp       505 --------------------igkg-i-irr----------
HIdxsp       494 --------------------igks-r-lir----------
PFdxsp      1020 ddvdkyseeymdddnfiksfigks-r-iikmdnennntne
SSdxsp       488 --------------------vggl-d-vlhrd--------
HPdxsp       485 --------------------lgqs-e-llk----------

STdxsp      1691 ----------eg--kk--vailslgtrlaealkaadtlea
AAdxsp       500 ----------eg--ed--cvilavgypvyqalraaeklyk
BSdxsp       500 ----------pg--nd--aviltfgttiemaieaaeelqk
CRdxsp       595 ----------qg--kd--vclvaygssvnealaaadmler
CJdxsp       492 ----------nn--se--iaflgygqgvakawqvlralqe
PAdxsp       507 ----------rg--gr--vallvfgvqlaeamkvaeslda
LEdxsp       584 ----------eg--er--vallgygsavqncldaaivles
MLdxsp       497 ----------tglaqd--vllvgvgvfasmalavakrlhn
MTdxsp       497 ----------dg--lnhdvllvaigafapmalavakrlhn
RCdxsp       505 ----------eg--te--vailsfgahlaqalkaaemlea
RSdxs1p      504 ----------eg--tr--iallsfgtrlaevqvaaealaa
RSdxs2p      507 ----------eg--td--vailsfgahlhealqaakllea
SPCCdxsp     503 ----------qg--dd--llmlaygsmvypalqtaellne
SPdxsp       504 ----------sg--dd--vlllgygsmvypalqtaellhe
TMdxsp       484 ----------rg--re--aaiiatgtilnevlkip-----
ECdxsp       502 ----------rg--ek--lailnfgtlmpeaakvaeslna
NMdxsp       513 ----------eg--ek--tafiafgsmvapalavagklna
HIdxsp       502 ----------kg--qk--iailnfgtllpsalelseklna
PFdxsp      1058 hyssrgdtqtkk--kk--vcifnmgsmlfnvinaikeiek
SSdxsp       498 ----------er--pe--vllvavgvmaqvclqtaellra
HPdxsp       493 ----------ke--ge--illigygngvgrahlvqlalke
```

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```
STdxsp        1769 k-----glsttvadlrfakpldedlirrll--tthevavt
AAdxsp         526 e-----girvgvvnarfvkpmdekmlrdla--nrydtfit
BSdxsp         526 e-----glsvrvvnarfikpidekmmksil--keglpilt
CRdxsp         621 d-----gvsttvidarfckpldtklirsaa--kehpvmit
CJdxsp         518 m-----nnnanlidlifakpldeellcela--kkskiwfi
PAdxsp         533 ----------tvvdmrfvkpldealvrela--gshellvt
LEdxsp         610 r-----glqvtvadarfckpldhalirsla--kshevlit
MLdxsp         525 q-----gigvtvidprwvlpvcdgvl-ela--hthklivt
MTdxsp         525 q-----gigvtvidprwvlpv-sdgvrela--vqhkllvt
RCdxsp         531 e-----gvsttvadarfcrpldtdlidrli--eghaalit
RSdxs1p        530 r-----gisptvadarfakpldrdlilqla--ahhealit
RSdxs2p        533 e-----gvsvtvadarfsrpldtghidqlv--rhhaalvt
SPCCdxsp       529 h-----gisatvinarfakpldeelivpla--rqigkvvt
SPdxsp         530 h-----gieatvvnarfvkpldtelilpla--erigkvvt
TMdxsp         505 -------ldvtvvnaltvkpldtavlkeia--rdhdliit
ECdxsp         528 ----------tlvdmrfvkpldealilema--ashealvt
NMdxsp         539 ----------tvadmrfvkpideelivrla--rshdrivt
HIdxsp         528 ----------tvvdmrfvkpidieminvla--qthdylvt
PFdxsp        1094 eqyishnysfsivdmiflnpldknmidhvikqnkhqylit
SSdxsp         524 r-----gigctvvdprwvkpv-dpvlppla--aehrlvav
HPdxsp         519 k-----niecalldlrflkpldpnlsaiva--pyqklyvf

STdxsp        1868 ieega-i-ggpgahv----ltlasdtglida-glklrtmr
AAdxsp         559 vednt-vvggfgsgv----leffaregimk----rvinlg
BSdxsp         559 ieeav-leggfgssi----lefahdqg--ey-htpidrmg
CRdxsp         654 ieegs-v-ggfaahv----mqflaleglldg-glkfrpmt
CJdxsp         551 fsenvki-ggiesli----nnflqk---ydl-hvkvvsfe
PAdxsp         561 ieena-vmggagsav----geflasegl----evpllqlg
LEdxsp         643 veegs-i-ggfgshv----vqfmaldglldg-klkwrpiv
MLdxsp         557 ledng-vnggvgaav----stalrq---vei-dtpcrdvg
MTdxsp         557 ledng-v-nggagsa----vsaalrraeid---vpcrdvg
RCdxsp         564 leqga-m-ggfgamv----lhylartgqlek-grairtmt
RSdxs1p        563 ieega-i-ggfgshv----aqllaeagvfdr-gfryrsmv
RSdxs2p        566 veqga-m-ggfgayv----mhclansggfdg-glalrvmt
SPCCdxsp       562 feegc-l---pggfg----saimeslqahdl-qvpvlpig
SPdxsp         563 meegc-lmggfgsav----aealmdnnvl----vplkrlg
TMdxsp         536 veeamki-ggfgsfv----aqrlqemgwqg----kivnlg
ECdxsp         556 veena-imggagsgvnevlmahrkpvpvlni-g-------
NMdxsp         567 leena-eqggagsav----levlakhgickp-vlll---g
HIdxsp         556 leena-iqggagsav----aevlnssgksta-llql---g
PFdxsp        1134 yednt-i-ggfsthf----nnyliennyitkhnlyvhniy
SSdxsp         556 vednsra-agvgsav----alalgda---dv-dvpvrrfg
HPdxsp         552 sdnyk-l-ggvasai----leflseqnilk----pvksfe
```

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```
STdxsdna      1967 lpdifqdqdkpekqydeaglnaanivdtvl-k-al-ryne
AAdxsp         590 vpdrfiehgkqdilrnlvgidaegiekavr-d-al-kggr
BSdxsp         591 ipdrfiehgsvtalleeigltkqqvanrir-l-lm----p
CRdxsp         687 lpdryidhgdyrdqlamagltsqhiastal-t-tlgrakd
CJdxsp         582 yedkfiehgkts----eveknlekdvnslltk-vl-kfyh
PAdxsp         592 lpdyyvehakpsemlaecgldaagiekavr-q-rl-drq-
LEdxsp         676 lpdryidhgspvdqlaeagltpshiaatvf-n-il-gqtr
MLdxsp         588 lpqefydhasrsevladlgltdqdvarrit-gwvv-afgh
MTdxsp         588 lpqefyehasrsevladlgltdqdvarrit-g-wv-----
RCdxsp         597 lpdcyidhgspeemyawagltandirdtal-a-aa-rpsk
RSdxs1p        596 lpdtfidhnsaevmyataglnaadierkal-e-tl---gv
RSdxs2p        599 lpdrfieqaspedmyadaglraediaatar-g-al-argr
SPCCdxsp       593 vpdllvehaspdeskqelgltprqmadril-e----kfgs
SPdxsp         594 vpdilvdhatpeqstvdlgltpaqmaqnim-a-sl-fkte
TMdxsp         567 vedlfvphggrkellsmlgldsegltktv-----l-tyik
ECdxsp         587 lpdffipqgtqeemraelgldaagmeaki----------k
NMdxsp         598 vadtvtghgdpkklldd1glsaeaverrvr-a-wl---sd
HIdxsp         587 lpdyfipqatqqealadlgldtkgieekil-n-fi-a-kq
PFdxsp        1168 lsnepiehasfkdqqevvkmdkcslvnrik-n-yl-knnp
SSdxsp         587 ipeqflaharrgevladigltpveiagrig-a-sl-pvre
HPdxsp         582 iidefimhgntalvekslgldtesltdail-k-dl-gqer

STdxsdna      2078 a----e--l--ad-----gvra*--------
AAdxsp         627 l----i-------------------------
BSdxsp         625 p----k--t--hk-----gigs---------
CRdxsp         725 a----a--kfsls-----alqa---------
CJdxsp         616 ---------------------------------
PAdxsp         628 ---------------------------------
LEdxsp         713 e----a--l--ev-----mt-----------
MLdxsp         626 c----g--s--gddagqygprssqtm-----
MTdxsp         621 a----a--l--gt-----gvcasdaipehld
RCdxsp         634 sv---r--i--vh-----sa-----------
RSdxs1p        631 e----v--l--ar-------ra---------
RSdxs2p        636 vmplrq--t--ak-----prav---------
SPCCdxsp       628 r----q--r--ig-----aasa---------
SPdxsp         631 t----esvv--ap-----gvs----------
TMdxsp         601 a----r--s--re-----gkv----------
ECdxsp         617 a----w--l--a--------------------
NMdxsp         633 r----d--a--an-------------------
HIdxsp         623 g----n--l----------------------
PFdxsp        1205 t----------------------------
SSdxsp         624 -----e--p--ae-----eqpa---------
HPdxsp         619 ---------------------------------
```

Figure 7

```
   1 cgacggcccg gtagccccgg cgcggctgca gcaccgtcag acgtccgccg
  51 agaaagccgt cggaagtcaa ttcgtccggg gcgaacatca gggggtcgtc
 101 gggatgccgt tgtcggacat cacccggcag gcgcgatccc agtcttcttc
 151 cgggacaaac agacgccgcg gcaatatgcc gatggagcct tcgaggacgc
 201 tcatgtggac gtccaccgga aaggcgtcta tatcctcgcc ctgaaggagc
 251 gcggtggcga aggcgatgat cgtcgggtcg gtcgtgcgca acagttcctt
 301 catgtcgggg acattgtcgg caacgcctcg gtttgtcgag gccggttcgt
 351 cgaccgggtg gcaggatcgg gatgggattg gacgaggttt cgcaaaagcc
 401 gcatgaacgg ctcgccgcgt ggctggccga ggacatggcc gccgtcaacg
 451 ggctgatccg cgagcggatg gcctcgaaac acgcgccccg cattcccgag
 501 gtcacggcgc atctggtcga ggccggcggc aagcggctgc ggccgctcct
 551 gacgctcgcc gcggcgcggc tgtgcggcta cgaggggccc tatcacatcc
 601 atctggccgc gacggtggag ttcatccaca cggcgacgct gcttcacgac
 651 gatgtggtgg acgaaagcca ccgccgccgc ggcaaaccca cggcgaacct
 701 gctgtgggac aacaaatcct cggtgctggt gggcgactat ctcttcgccc
 751 gcagcttcca gctgatggtc gagaccggct cgcttcgcgt gatggacatc
 801 ctcgccaatg cctcggccac catctccgag ggcgaggtgc tgcagctgac
 851 cgcggcccag gatctgcgca cgaccgagga catccacctg caggtggtgc
 901 gcggcaagac ggccgcgctc tttgccgcgg caaccgaggt gggcggcgtg
 951 gtcgcgggcg tgcccgaggc gcaggtcgag gcgctccacg cctacgggga
1001 cgcgctgggg atcgccttcc agatcgtcga cgacctcctc gattatggcg
1051 gcgtggatgc ccagatcggc aagaacaccg gcgacgactt ccgcgaacgc
1101 aagctgacgc tgccggtcat caaggcggtg gcccaggccg atgccgagga
1151 gcgcgccttc tggcagcggg tgatcgagaa gggcgaccag cgcgagggtg
1201 acctcgagca agcccatgcg atcatgtccc gccacggcgc catggaggcc
1251 gcccggcagg atgcgctccg ctgggtcacg gtggcgcgcg aggcactcgg
1301 ccagctgccg gagcacccgc tgcgcgagat gctgcacgat ctggccgatt
1351 tcgtggtcga acgcatcgcc tgatcccttc cgggcgctct gccccggcgc
1401 agcgcaggat cccgcgctgc gccccctttcg gccttccgac agtccctctg
1451 ccgcgggagg ccggcctcgc ctgagaagcc gcactggccg ccggtcttcc
1501 cccgaaccgc tcccgggcct gctcggaagg cgtccgccgc aaaagccccc
1551 gcgggggggc cccaccggcg gccatcagga agagaccgtt gaagcggccc
1601 gctcgaatcc tgtcgcgccc ccccccgacc gggcggctct ccgatccgtg
1651 ttcgctcggc gatggacagc cgttccctgt ccgttcatga tggcgccatg
1701 cagaccctta ccgttcccga ttccggcctc gccccctcct gcccggccaa
1751 aggctcgccc gcggcgtctg ccgccatctg cgcagccatg atttcgtctc
1801 ggtggtcgaa ctcgtgcccg cgcccggcct cagggtcgac gtgatggcgc
1851 tggggcccaa gggcgagatc tgggtggtgg aatgcaaatc ctcgcgcgcg
1901 gactatcagt ccgaccgcaa gtggcagggc tatctcgact ggtgcgaccg
1951 cttcttcttc gcggtggacg aggaccagcc cgggccgtcg  (SEQ ID
NO:37)
```

Figure 8

```
   1  atgggattgg acgaggtttc gcaaaagccg catgaacggc tcgccgcgtg
  51  gctggccgag gacatggccg ccgtcaacgg gctgatccgc gagcggatgg
 101  cctcgaaaca cgcgccccgc attcccgagg tcacggcgca tctggtcgag
 151  gccggcggca agcggctgcg gccgctcctg acgctcgccg cggcgcggct
 201  gtgcggctac gaggggccct atcacatcca tctggccgcg acggtggagt
 251  tcatccacac ggcgacgctg cttcacgacg atgtggtgga cgaaagccac
 301  cgccgccgcg gcaaacccac ggcgaacctg ctgtgggaca acaaatcctc
 351  ggtgctggtg ggcgactatc tcttcgcccg cagcttccag ctgatggtcg
 401  agaccggctc gcttcgcgtg atggacatcc tcgccaatgc ctcggccacc
 451  atctccgagg gcgaggtgct gcagctgacc gcggcccagg atctgcgcac
 501  gaccgaggac atccacctgc aggtggtgcg cggcaagacg gccgcgctct
 551  ttgccgcggc aaccgaggtg ggcggcgtgg tcgcgggcgt gcccgaggcg
 601  caggtcgagg cgctccacgc ctacggggac gcgctgggga tcgccttcca
 651  gatcgtcgac gacctcctcg attatggcgg cgtggatgcc cagatcggca
 701  agaacaccgg cgacgacttc cgcgaacgca agctgacgct gccggtcatc
 751  aaggcggtgg cccaggccga tgccgaggag cgcgccttct ggcagcgggt
 801  gatcgagaag ggcgaccagc gcgagggtga cctcgagcaa gcccatgcga
 851  tcatgtcccg ccacggcgcc atggaggccg cccggcagga tgcgctccgc
 901  tgggtcacgg tggcgcgcga ggcactcggc cagctgccgg agcacccgct
 951  gcgcgagatg ctgcacgatc tggccgattt cgtggtcgaa cgcatcgcct
1001  ga  (SEQ ID NO:38)
```

Figure 9

```
  1  mgldevsqkp herlaawlae dmaavnglir ermaskhapr ipevtahlve
 51  aggkrlrpll tlaaarlcgy egpyhihlaa tvefihtatl lhddvvdesh
101  rrrgkptanl lwdnkssvlv gdylfarsfq lmvetgslrv mdilanasat
151  isegevlqlt aaqdlrtted ihlqvvrgkt aalfaaatev ggvvagvpea
201  qvealhaygd algiafqivd dlldyggvda qigkntgddf rerkltlpvi
251  kavaqadaee rafwqrviek gdqregdleq ahaimsrhga meaarqdalr
301  wvtvarealg qlpehplrem lhdladfvve ria (SEQ ID NO:39)
```

Figure 10

```
   1  ggatcgcgca gcgcctcggc cacgcgcacc atcagcagca gattgccgtt
  51  cggcagccgc gcgaagccgg ggttgaaggc gccaaggaca taggtcgcgt
 101  cgtccacccc ctcgcgcagc ggtgagcggg tcaggtcgac attgtcgggc
 151  cggaagatca gataatcgtc gctcaagcgc ttgccccctc gggtttcacg
 201  cccagcaacg gggtcaggcc ccgggggttc cggcttcagc gccggcttcc
 251  tgggcctggc ggtggtgccg gatcacctcg tcgatgatga agcgcaggaa
 301  tttctcggaa aattcggggt cgagatcggc atcctgcgcc agcgcgcgca
 351  gccgggcgat ctgcgcctcc tcgcggccgg gatcggcggg cggcagcccg
 401  gattcggcct tgtagcgccc caccgcctgg gtcaccttga accgctcggc
 451  gagcatgaag acgagcgccg catcgatatt gtcgatgctc tggcgatagc
 501  gggtcagcgt cgcgtcggtc atgcgaatct cctttgccgc tgcggcacgg
 551  ccatgcaagc acctcttgcc tttgcaatgc acaaaggcca gaggctcgtt
 601  gcatatgagc gcaaccgtcc accgcctggg ctcgcgaacc cagccttcgc
 651  tcgatccgat catggcgctg gtcgcccagg acatgaacct ggtgaacgcg
 701  gtgatcctcg atcgcatgca gtccgagatc ccgctgatcc ccgaactcgc
 751  cggccatctg atcgctggcg gcggcaagcg gatgcggccg atgctgacgc
 801  tcgccagcgc ccggctgctc ggctattcgg gcacgcgcca ccacaagctg
 851  gcggcggcag tggagttcat ccacaccgcg acgctgctgc atgacgacgt
 901  ggtcgacagc tcggacctgc gccgcggccg ccgcaccgcc aacatcatct
 951  ggggcaatcc cgccagcgtg ctggtcggcg acttcctgtt cagccgctcg
1001  ttcgagctga tggtcgaggc cgaaagcctc aaggcgctgc acatcctgtc
1051  gaacgccagc gcggtgatcg ccgagggcga agtcaaccag ctgaccgcgg
1101  tgcgccggat cgacctgtcc gaggatcgct atctcgacat catcggcgcc
1151  aagactgcgg cgctgttcgc cgccgcctgc cgggtggcgg gcgtggtcgc
1201  cgagcgtccc gaggcggagg aactcgcgct cgacgcctat ggccgcaacc
1251  tcggcatcgc tttccagctg gtcgacgacg cgatcgacta tgtctcggac
1301  gcgtcgacga tgggcaagga tgccggcgac gatttccgcg aaggcaagat
1351  gacgctgccg gtggtcctgg cgtacgcgcg cggcgacgag gcggaacgcg
1401  gcttctggaa ggaagcgatt tcgggccgcc gcatctcgga cgaggatttc
1451  gccgaggcga tccggctggt gcagagctgc cgcgcggtgg acgacacgct
1501  cgcccgtgcc cgccattacg gccagctcgc gatcgatgcg ctgggcggct
1551  tccgcgcctg cgaggcgaag gacgcgatgg tcgaggcggt cgaattcgcg
1601  gtggcgcgcg cctactgacg cgcgccgacc ggagcatttc cgggtggatc
1651  gcttgcgatc caaggctcgg gaaatgcgac catcaaaaag cttccgggga
1701  ttacgcctcg gtcgactttt cttcgccctc gtcctcgtcg acttcgagcg
1751  cgtcttcctc gtccatgtcg agcactacct cgatgccctc gacgatcagg
1801  tcgagctgct cgtagctcgc cgtcatctcg atc  (SEQ ID NO:40)
```

Figure 11

```
   1  atgagcgcaa ccgtccaccg cctgggctcg cgaacccagc cttcgctcga
  51  tccgatcatg gcgctggtcg cccaggacat gaacctggtg aacgcggtga
 101  tcctcgatcg catgcagtcc gagatcccgc tgatccccga actcgccggc
 151  catctgatcg ctggcggcgg caagcggatg cggccgatgc tgacgctcgc
 201  cagcgcccgg ctgctcggct attcgggcac gcgccaccac aagctggcgg
 251  cggcagtgga gttcatccac accgcgacgc tgctgcatga cgacgtggtc
 301  gacagctcgg acctgcgccg cggccgccgc accgccaaca tcatctgggg
 351  caatcccgcc agcgtgctgg tcggcgactt cctgttcagc cgctcgttcg
 401  agctgatggt cgaggccgaa agcctcaagg cgctgcacat cctgtcgaac
 451  gccagcgcgg tgatcgccga gggcgaagtc aaccagctga ccgcggtgcg
 501  ccggatcgac ctgtccgagg atcgctatct cgacatcatc ggcgccaaga
 551  ctgcggcgct gttcgccgcc gcctgccggg tggcgggcgt ggtcgccgag
 601  cgtcccgagg cggaggaact cgcgctcgac gcctatggcc gcaacctcgg
 651  catcgctttc cagctggtcg acgacgcgat cgactatgtc tcggacgcgt
 701  cgacgatggg caaggatgcc ggcgacgatt tccgcgaagg caagatgacg
 751  ctgccggtgg tcctggcgta cgcgcgcggc gacgaggcgg aacgcggctt
 801  ctggaaggaa gcgatttcgg gccgccgcat ctcggacgag gatttcgccg
 851  aggcgatccg gctggtgcag agctgccgcg cggtggacga cacgctcgcc
 901  cgtgcccgcc attacggcca gctcgcgatc gatgcgctgg gcggcttccg
 951  cgcctgcgag gcgaaggacg cgatggtcga ggcggtcgaa ttcgcggtgg
1001  cgcgcgccta ctga  (SEQ ID NO:41)
```

Figure 12

```
  1  msatvhrlgs rtqpsldpim alvaqdmnlv navildrmqs eiplipelag
 51  hliagggkrm rpmltlasar llgysgtrhh klaaavefih tatllhddvv
101  dssdlrrgrr taniiwgnpa svlvgdflfs rsfelmveae slkalhilsn
151  asaviaegev nqltavrrid lsedryldii gaktaalfaa acrvagvvae
201  rpeaeelald aygrnlgiaf qlvddaidyv sdastmgkda gddfregkmt
251  lpvvlayarg deaergfwke aisgrrisde dfaeairlvq scravddtla
301  rarhygqlai dalggfrace akdamveave favaray (SEQ ID NO:42)
```

Figure 13 (page 1 of 5)

```
RSddsdna      372 atg----------------------------ggattggac
STddsdna      605 atg----------------------------agcgcaacc
SPddsdna        1 atgattcagtatgtatatttaaaacatatgaggaaattat
GSddsdna        1 ------------------------------------
RCddsdna        1 atg----------------------------gccatcga-

RSddsdna      384 ga---------ggtttcgcaaaagccgcat------gaac
STddsdna      617 gtccaccgcctgggctcgcgaacccagccttcgctcgatc
SPddsdna       41 gg---------agtcttggaaaagtccgtt------cgac
GSddsdna        1 ------------------------------------
RCddsdna       12 -------------tttc---aa----gcaa------gata

RSddsdna      409 ggctcgccgcgtggctggccgaggacatggccgccgtca-
STddsdna      657 cgatcatggcgctggtcgcccaggacatgaacctggtga-
SPddsdna       66 tgttcttcggttttct--actacgaaccgcaatgcttcac
GSddsdna        1 -------------------------atgctggcctgca-
RCddsdna       26 ttctcg-ctcctg--ttgctcaagattttgcagcgatgg-

RSddsdna      448 acgggctgatccgcgagcggatggcctcgaaaca---cgc
STddsdna      696 acgcggtgatcctcgatcgcatgcagtccgagat---c--
SPddsdna      104 atttaattaaaaacgag---------ttggaacaaatctc
GSddsdna       14 accgggcgatcatcgcccggatg-----gaaagt---ccg
RCddsdna       62 accagtttattaatgaaggaatcagctccaaggt---cgc

RSddsdna      485 g--ccccgcattc--------ccgaggtca----cggcgc
STddsdna      731 ---ccgctgatcc--------ccgaactcg----ccggcc
SPddsdna      135 a--ccagggattcgtcaaatgctgaattcaaattcagaat
GSddsdna       46 gttcccctgatcc--------cgcagcttg----gcgccc
RCddsdna       99 a--ctggtcatgt--------c---agtca----gcaagc

RSddsdna      511 atctggtcgag---------------------gccggcgg
STddsdna      756 atctgatcgct---------------------ggcggcgg
SPddsdna      173 ttcttgaagagtgttctaaatattataccattgctcaagg
GSddsdna       74 atcttgtcgcg---------------------gcgggagg
RCddsdna      122 atgtcgttgaa---------------------gcaggtgg

RSddsdna      530 caagcggctgcggccgc---------tcctgacgctcgcc
STddsdna      775 caagcggatgcggccga---------tgctgacgctcgcc
SPddsdna      213 aaaacaaatgcgtccttctcttgttttgctgatgtccaaa
GSddsdna       93 caagcgccttcgcccgc---------tgctgacgctggcc
RCddsdna      141 aaagcgcatgcgtccga---------ttatg-tgcttgct
```

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```
RSddsdna    561 gcggcgcggctgtgc---ggctacgag--gggccc-----
STddsdna    806 agcgcccggctgctc---ggctattcg--ggcacg-----
SPddsdna    253 gctacaagcttgtgccatggtattgat--cggtccgtagt
GSddsdna    124 tccgcacgtctgtgc---ggttatcagccgggtcc-----
RCddsdna    171 g----gccgct-tat---gcctgtggt--gaaacc-----

RSddsdna    591 ----------------------------t----atcacatc
STddsdna    836 ----------------------------c----gccaccac
SPddsdna    291 gggcgacaaatatattgatgatgatgat----ttaagatc
GSddsdna    156 ----------------------------ggaccatcagcgt
RCddsdna    196 ----------------------------a----atttaaag

RSddsdna    600 cat---------ctggccgcgacggtg-------------
STddsdna    845 aag---------ctggcggcggcagtg-------------
SPddsdna    327 att---------ttcgacgggtcaaattcttccttctcaa
GSddsdna    169 catgtcggg---ctcgccgcctgcgtt-------------
RCddsdna    205 catgcacagaagctggcggccattatt-------------

RSddsdna    618 ----------------------gagttcatccacacggcga
STddsdna    863 ----------------------gagttcatccacaccgcga
SPddsdna    358 ttgagattagcacaaataaccgagatgatccatatagcaa
GSddsdna    193 ----------------------gagttcattcataccgcca
RCddsdna    232 ----------------------gaaatgctgcatacggcga

RSddsdna    637 cgctgcttcacgacgatgtggtggacgaaagccaccgccg
STddsdna    882 cgctgctgcatgacgacgtggtcgacagctcggacctgcg
SPddsdna    398 gtttgctgcatgacgatgtgattgatcacgctaatgtccg
GSddsdna    212 cactgctgcatgatgatgtcgtggatgagagcacgttgcg
RCddsdna    251 ctctggtacatgatgatgatgtagatgagtctggcttacg

RSddsdna    677 ccgcggcaaacccacg-gcgaacctgctgtgggacaacaa
STddsdna    922 ccgcggccgccgcacc-gccaacatcatctggggcaatcc
SPddsdna    438 tagaggctcaccttcaagcaatgttgctttcgg-----ta
GSddsdna    252 tcgggggctggcttcg-gccaatgccgtgttcggcaacaa
RCddsdna    291 ccgtggcagaccaaca-gcaaatgcgacatggaataacca

RSddsdna    716 atcctcggtg----ctggtgggcgactatctcttcgcccg
STddsdna    961 cgccagcgtg----ctggtcggcgacttcctgttcagccg
SPddsdna    473 atcgacggtcaatccttgcgggtaatttcatccttgcacg
GSddsdna    291 ggcgtccgtg----ctggtaggtgacttcctgttcgcccg
RCddsdna    330 gactgcggta----ctggtgggggattttctgattgcccg
```

Figure 13 (page 3 of 5)

```
RSddsdna     752 cagcttccagctgatggtcgagaccggctcg-----cttc
STddsdna     997 ctcgttcgagctgatggtcgaggccgaaagc-----ctca
SPddsdna     513 g-gcttcga----ctgctatggcccgccttcgaaatcccc
GSddsdna     327 ctcgttccagcttatgacagcagacggctcc-----ctga
RCddsdna     366 ggcatttgatctgctggttgatctggacaat-----atga

RSddsdna     787 gcgtgatggacatcctcgccaatgcctcggccaccatctc
STddsdna    1032 aggcgctgcacatcctgtcgaacgccagcgcggtgatcgc
SPddsdna     548 aagttacggagttgttagctacagtgatagcagacttggt
GSddsdna     362 aggtcatggcgatcctgtcggatgcatcggcgacaattgc
RCddsdna     401 tcctgttaaaggacttctctacaggaacctgtgagattgc

RSddsdna     827 cgagggcgaggtgctgcagctgaccgcgg--cccaggatc
STddsdna    1072 cgagggcgaagtcaaccagctgaccgcggtgcgccggatc
SPddsdna     588 tcgaggtgagtttttgcagctaaaaaata--ctatggat-
GSddsdna     402 tgaaggtgaagtccttcagatggtcgtgc--agaacgacc
RCddsdna     441 tgagggtgaagtattgcagttgc---agg--cacagcatc

RSddsdna     865 tgcgc---acgaccgaggacatccacc-------------
STddsdna    1112 --gac---ctgtccgaggatcgctatc-------------
SPddsdna     625 --cct---tcatctttggaaataaaacaatcaaattttga
GSddsdna     440 ttacg---acgcctgtagaacgctatc-------------
RCddsdna     476 agccagatacaacagaagatatttatt-------------

RSddsdna     889 ---------tgcaggtggtgcgcggcaagacggccgcgct
STddsdna    1134 ---------tcgacatcatcggcgccaagactgcggcgct
SPddsdna     660 ctattatattgaaaaaagttttttg-aaaacagccagttt
GSddsdna     464 ---------ttgaagtcattcacggcaagacggctgcgct
RCddsdna     503 ---------tacagattattcacggtaaaacctcacggtt

RSddsdna     920 ctttgccgcggcaaccgaggtgggcggcgtggtcg-----
STddsdna    1165 gttcgccgccgcctgccgggtggcgggcgtggtcg-----
SPddsdna     699 aatttcca-------aaagctgcaaggcttctacaatcct
GSddsdna     495 gtttgcggctgcctgccgtgtcggcgctgtcgtgg-----
RCddsdna     534 gttcgaactggcgaccgaaggcgctgcaatactgg-----

RSddsdna     955 cgggc---gtgcccgaggcgcaggtcgaggcgctccacgc
STddsdna    1200 ccgag---cgtcccgaggcggaggaactcgcgctcgacgc
SPddsdna     732 cggacaatgttctcctactgtagcaacagctgctgga-ga
GSddsdna     530 ccgag---cgtccggaagcagaagaggaagctctggagcg
RCddsdna     569 caggc---aaacctga-----ataccgtgaacctttacgt
```

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```
RSddsdna     992 c---tacggggacgcgctggggatcgccttccagatcgtc
STddsdna    1237 c---tatggccgcaacctcggcatcgctttccagctggtc
SPddsdna     771 a---tacggtcgatgcattggtactgcttttcaactaatg
GSddsdna     567 g---tttggcaccaatctgggtatggcgttccagcttgtt
RCddsdna     601 cgttttgccggacactttggcaat-gcttttcagattatt

RSddsdna    1029 gacgacctcctcgattatggcggcgtg-gatgcccagatc
STddsdna    1274 gacgacgcgatcgactatgtctcggac-gcgtcgacgatg
SPddsdna     808 gatgacgtgttggactat-acgtcgaaagatgatacttta
GSddsdna     604 gatgatgccctggattatgccgcagac-cagcaggttttg
RCddsdna     640 gatgatattctggattacacttcagat-gctgatacgctc

RSddsdna    1068 ggcaagaacaccggcgacgacttcc-gcgaacgcaagctg
STddsdna    1313 ggcaaggatgccggcgacgatttcc-gcgaaggcaagatg
SPddsdna     847 ggaaaggcggctggtgcagatttgaagctagggttggcta
GSddsdna     643 ggcaagaccgttggtgatgacatgc-gtgaaggcaagatc
RCddsdna     679 ggcaaaaatattggcgatgacttga-tggaaggcaaaccc

RSddsdna    1107 acgctgccggtcatcaaggcggtggcccaggccgatgcc-
STddsdna    1352 acgctgccggtggtcctggcgtacgcgcgcggcgacgag-
SPddsdna     887 cagct-cccgtcctctttgc-atggaaaaagt--atcca-
GSddsdna     682 accctgccggtcct------ggccgcctatgaggctggct
RCddsdna     718 accctgccgctgattgcagcaatgcaaaatactcaaggt-

RSddsdna    1146 -----gaggagcgcgccttctggcagcgggtgatcgagaa
STddsdna    1391 -----gcggaacgcggcttctggaaggaagcgatttcg--
SPddsdna     922 -----ga--------acttggtgca----atgattgtgaa
GSddsdna     716 cgccggaagatcgtattttctgggagcgcgtcattggaga
RCddsdna     757 -----gaacagcgcgacctgatccgtcgc-------agca

RSddsdna    1181 gggcgaccagcgcgagggtgac--ctcgagcaagcccatg
STddsdna    1424 ----ggccgccgcatctcggac--gaggatttcgccgagg
SPddsdna     945 tagattcaatcatccttctgat--atccaacgggctcgtt
GSddsdna     756 aggggagcagactgaggacgat--ctgcctcatgctctga
RCddsdna     785 ttgccactggcg-gtacttcacagcttgaacaagttattg

RSddsdna    1219 cgatca------tgtcccgccacggcgccatggaggc--c
STddsdna    1458 cgatccggctggtgcagagctgccgcgcggtggacga--c
SPddsdna     983 ctttgg------ttgagtgcactgatgctatcgagca--a
GSddsdna     794 acctga------ttgcaaagacgggtgcgatcaatacgac
RCddsdna     824 cgattg------tacaaaattcgggagcgctgga------
```

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```
RSddsdna      1251 gcccggcaggatgcgctccgctgggtcacggtggcgcgcg
STddsdna      1496 acgctcgcccgtgcccgccattacggccagctcgcgatcg
SPddsdna      1015 accatcacttgggcaaaagaatatatcaaaaaagccaaag
GSddsdna       828 gatcgcccg--cgcgcaggtctatgccgacgcagctgttg
RCddsdna       852 ttattgccataagcgtgctactgaagaaaccgagcgagca

RSddsdna      1291 ----aggcactcggccagctgccggagcacccgctgcgcg
STddsdna      1536 ----atgcgct-gggcggcttcc-gcgcctgcgaggcgaa
SPddsdna      1055 ----attcccttctgtgtctccctgattcacctgcaagga
GSddsdna       866 ----aagccctgtccattttcccggatagcgaactgcgcc
RCddsdna       892 ttacaggcactagaaatattacctgagagtacttaccggc

RSddsdna      1327 agatgc--tgcacgatctggccgatttcgtggtcgaacgc
STddsdna      1570 ggacgcgatggtcgaggcggtcgaattcgcggtggcgcgc
SPddsdna      1091 aggcac--tttttgcgttggctgataaagtaataacgaga
GSddsdna       902 gccttc--tgatcgaaacggttcagttcacggtgaatcgg
RCddsdna       932 aggcgc--tggttaacttgacccgcttagctttagaccga

RSddsdna      1365 atcgcctga
STddsdna      1610 gcctactga
SPddsdna      1129 aagaagtga
GSddsdna       940 gcccgctaa
RCddsdna       970 atccaataa
```

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```
RSddsp        372 ------------mgldevsq-----kphe
STddsp        605 msatv--------hrlgsrtq-----psld
SPddsp          1 miqyvylkhmrklwslgkvrstvlrfsttn
GSddsp          1 ----------------------------
RCddsp          1 ------------maidf----------kq

RSddsp        408 rlaawlae-dmaavnglirermaskhapri
STddsp        656 pimalvaq-dmnlvnavildrmqse-ipli
SPddsp         31 rnashlikneleqispgirq-mlnsnsefl
GSddsp          1 ----------mlacnraiiarmesp-vpli
RCddsp          8 dilapvaq-dfaamdqfinegisskva-lv

RSddsp        495 pevtahlveaggkrlrplltla---aarlc
STddsp        740 pelaghliagggkrmrpmltla---sarll
SPddsp         60 eecskyytiaqgkqmrpslvllmskatslc
GSddsp         20 pqlgahlvaaggkrlrplltla---sarlc
RCddsp         36 msvskhvveaggkrmrpimcll---aayac

RSddsp        576 ----------------------gye-gp-
STddsp        821 ----------------------gys-gt-
SPddsp         90 hgidrsvvgdkyiddddlrsfstgqi-lp-
GSddsp         47 ----------------------gyqpgpd
RCddsp         63 ----------------------get-nl-

RSddsp        591 --yhih-laatvefihtatllhddvvdesh
STddsp        836 --rhhk-laaavefihtatllhddvvdssd
SPddsp        118 --sqlr-laqitemihiasllhddvidhan
GSddsp         54 hqrhvg-laacvefihtatllhddvvdest
RCddsp         68 --khaqklaaiiemlhtatlvhdddvdesg

RSddsp        672 rrrgkptanllwdnkssvlvgdylfarsfq
STddsp        917 lrrgrrtaniiwgnpasvlvgdflfsrsfe
SPddsp        145 vrrgspssnvafgnrrsilagnfilarast
GSddsp         83 lrrglasanavfgnkasvlvgdflfarsfq
RCddsp         96 lrrgrptanatwnnqtavlvgdfliarafd

RSddsp        762 lmvetgslrvmdilanasatisegevlqlt
STddsp       1007 lmveaeslkalhilsnasaviaegevnqlt
SPddsp        175 amarlrnpqvtellatviadlvrgeflqlk
GSddsp        113 lmtadgslkvmailsdasatiaegevlqmv
RCddsp        126 llvdldnmillkdfstgtceiaegevlqlq
```

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```
RSddsp        852 aaqdlrtte------dihlqvvrgktaalf
STddsp       1097 avrridlse------dryldiigaktaalf
SPddsp        205 ntmdpssleikqsnfdyyieksflktasli
GSddsp        143 vqndlttpv------erylevihgktaalf
RCddsp        156 aqhqpdtte------diylqiihgktsrlf

RSddsp        924 aaatevggvvagvpeaqvealhaygdalgi
STddsp       1169 aaacrvagvvaerpeaeelaldaygrnlgi
SPddsp        235 sksckastilgqcsptvataageygrcigt
GSddsp        167 aaacrvgavvaerpeaeeealerfgtnlgm
RCddsp        180 elategaailagkpeyr-eplrrfaghfgn

RSddsp       1014 afqivddlldyggvdaqigkntgddfrerk
STddsp       1259 afqlvddaidyvsdastmgkdagddfregk
SPddsp        265 afqlmddvldytskddtlgkaagadlklgl
GSddsp        197 afqlvddaldyaadqqvlgktvgddmregk
RCddsp        209 afqiiddildytsdadtlgknigddlmegk

RSddsp       1104 ltlpvikavaqadaeerafwqrviekgdq-
STddsp       1349 mtlpvvlayargdeaergfwkeaisgrri-
SPddsp        295 atapvlfa----------wkkypelgami
GSddsp        227 itlpvlaayeagspedrifwervigegeq-
RCddsp        239 ptlpliaamqntqgeqrdlirrsiatggt-

RSddsp       1191 -----regdleqahaimsrhgameaarqda
STddsp       1436 -----sdedfaeairlvqscravddtlara
SPddsp        314 vnrfnhpsdiqrarslvectdaieqtitwa
GSddsp        256 -----teddlphalnliaktgainttiara
RCddsp        268 -----sq--leqviaivqnsgaldychkra

RSddsp       1266 lrwvtvarealgqlpehplremlhdladfv
STddsp       1511 rhygqlaidalggfraceakdamveavefa
SPddsp        344 keyikkakdsllclpdsparkalfaladkv
GSddsp        281 qvyadaavealsifpdselrrllietvqft
RCddsp        291 teeteralqaleilpestyrqalvnltrla

RSddsp       1356 veria*
STddsp       1601 varay*
SPddsp        374 itrkk-
GSddsp        311 vnrar-
RCddsp        321 ldriq-
```

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```
Hidxsp   1 mtnnmnnypllslinspedlrllnkdqlpqlcqelrayllesvsqtsghl
Ecdxsp   1 msfdiakyptlalvdstqelrllpkeslpklcdelrrylldsvsrssghf
Hpdxsp   1 ----------milqnktfdlnpndiaglelvcqtlrnrilevvsangghl

Hidxsp  51 asglgtveltvalhyvyktpfdqliwdvghqayphkiltgrreqmstirq
Ecdxsp  51 asglgtveltvalhyvyntpfdqliwdvghqayphkiltgrrdkigtirq
Hpdxsp  41 ssslgavelivgmhalfdcqknpfifdtshqayahkllтgrfesfstlrq

Hidxsp 101 kdgihpfpwreesefdvlsvghsstsisaglgiavaaerenagrktvcvi
Ecdxsp 101 kgglhpfpwrgeseydvlsvghsstsisagigiavaaekegknrrtvcvi
Hpdxsp  91 fqglsgftkpsesaydyfiaghsstsvsigvgvakafrlkqtlgmpiall

Hidxsp 151 gdgaitagmafealnhagalhtdmlvilndnemsisenvgalnnhlarif
Ecdxsp 151 gdgaitagmafeamnhagdirpdmlvilndnemsisenvgalnnhlaqll
Hpdxsp 141 gdgsisagifyealnelgdrkypmimilndnemsistpigalskalsqlm

Hidxsp 201 sgslystlrdgskkildkvppiknfm-kkteehmkgvmfspestlfeelg
Ecdxsp 201 sgklysslreggkkvfsgvppikell-krteehikgmvv--pgtlfeelg
Hpdxsp 191 kgpfyqsfrskvkkilstlpesvnylasrfeesfk--litp-gvffeelg

Hidxsp 250 fnyigpvdghnidelvatltnmrnlkgpqflhiktkkgkgyapaekdpig
Ecdxsp 248 fnyigpvdghdvlglittlknmrdlkgpqflhimtkkgrgyepaekdpit
Hpdxsp 238 inyigpinghdlgtiietlklakelkepvlihaqtlkgkgykiaegryek

Hidxsp 300 fhgvpkfdpisgelpknnsk-ptyskifgdwlcemaekdakiigitpamr
Ecdxsp 298 fhavpkfdpssgclpkssgglpsyskifgdwlcetaakdnklmaitpamr
Hpdxsp 288 whgvgpfdldtglskksksatlspteaysntllelakkdekivgvtaamp

Hidxsp 349 egsgmvefsqrfpkqyfdvaiaeqhavtfatglaiggykpvvaiystflq
Ecdxsp 348 egsgmvefsrkfpdryfdvaiaeqhavtfaaglaiggykpivaiystflq
Hpdxsp 338 sgtgldklidayplrffdvaiaeqhaltsssamakegfkpfvsiystflq

Hidxsp 399 raydqlihdvaiqnlpvlfaidragivgadgathqgafdisfmrcipnmi
Ecdxsp 398 raydqvlhdvaiqklpvlfaidragivgadgqthqgafdlsylrcipemv
Hpdxsp 388 raydsivhdacisslpiklaidragivgedgethqglldvsylrsipnmv

Hidxsp 449 imtpsdenecrqmlytgyqcgk-paavryprgn-avgvkltplemlpigk
Ecdxsp 448 imtpsdenecrqmlytgyhyndgpsavryprgn-avgveltpleklpigk
Hpdxsp 438 ifaprdnetlknavyfanehdsspcafryprgsfalkegvfepsgfvlgr
```

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```
Hidxsp 497 srlirkgqkiailnfgtllpsa--lelsek---lnatvvdmrfvkpidie
Ecdxsp 497 givkrrgeklailnfgtlmpea--akvaes---lnatlvdmrfvkpldea
Hpdxsp 488 sellkkegeilligygngvgrahlvqlalkekniecalldlrflkpldhn

Hidxsp 542 minvlaqthdylvtleenaiqggagsavaevlnssgkstallqlglpdyf
Ecdxsp 542 lilemaashealvtveenaimggagsgvnevlmahrkpvpvlniglpdff
Hpdxsp 538 l-saiiapyqklyvfsdnyklggvasaileflseqnilkpvksfeitdef

Hidxsp 592 ipqatqqealadlgldtkgieekilnfiakqgnl
Ecdxsp 592 ipqgtqeemraelgldaagmeakikawla-----
Hpdxsp 587 imhgntalvekslgldtesltdailkdlgqer--
```

Figure 16

```
Rpodsp   1 --mniivkiqqnlkdevtqlndliisclksdaeliekvgkylveaggkri
Ecoppp   1 mnlekinel---taqdmagvnaaileqlnsdvqlinqlgyyivsgggkri
Gsddsp   1 ----------------mlacnraiiarmespvplipqlgahlvaaggkrl
Rcsdsp   1 maidfkqdilapvaqdfaamdqfinegisskvalvmsvskhvveaggkrm

Rpodsp  49 rplltiitakmfdykgn----nhiklasavefihaatllhddvvdnstlr
Ecoppp  48 rpmiavlaaravgyegna----hvtiaaliefihtatllhddvvdesdmr
Gsddsp  35 rplltlasarlcgyqpgpdhqrhvglaacvefihtatllhddvvdestlr
Rcsdsp  51 rpimcllaayacg-etnlkhaqk--laaiiemlhtatlvhddvvdesglr

Rpodsp  95 rfkptanviwgsktsilvgdflfsqsfklmvasgcikamnvlakasviis
Ecoppp  94 rgkatanaafgnaasvlvgdfiytrafqmmtslgslkvlevmseavnvia
Gsddsp  85 rglasanavfgnkasvlvgdflfarsfqlmtadgslkvmailsdasatia
Rcsdsp  98 rgrptanatwnnqtavlvgdfliarafdllvdldnmillkdfstgtceia

Rpodsp 145 egevvqlvklnerriitideyqqivksktaelfgaacevgaiiaeqvdrv
Ecoppp 144 egevlqlmnvndpdi-teenymrviysktarlfeaaaqcsgilagctpee
Gsddsp 135 egevlqmvvqndltt-pverylevihgktaalfaaacrvgavvaerpeae
Rcsdsp 148 egevlqlqaqhqpdt-tediylqiihgktsrlfelategaailagkpe-y

Rpodsp 195 skdvqnfgrllgtifqviddlldylgsdkqvgknigddflegkvtlplif
Ecoppp 193 ekglqdygrylgtafqliddlldynadgeqlgknvgddlnegkptlpllh
Gsddsp 184 eealerfgtnlgmafqlvddaldyaadqqvlgktvgddmregkitlpvla
Rcsdsp 196 replrrfaghfgnafqiiddildytsdadtlgknigddlmegkptlplia

Rpodsp 245 lyhkleqdkqlwlenmlksdk--rtkddfvkirdlmlkhaiynetvnyls
Ecoppp 243 amhhgtpeqaqmirtaieqgngrhllepvleamnac---gslewtrqrae
Gsddsp 234 ayeagspedrifwervi--gegeqteddlphalnliaktgainttiaraq
Rcsdsp 246 amqntqgeqrdlirrsiatggtsqle----qviaivqnsgaldychkrat

Rpodsp 293 sleneannllnkipvqniykyylfsiirfilyrsy
Ecoppp 290 eeadkaiaalqvlpdtpw-realiglahiavqrdr
Gsddsp 282 vyadaavealsifpdsel-rrllietvqftvnrar
Rcsdsp 292 eeteralqaleilpesty-rqalvnltrlaldriq
```

Figure 17

```
Rpodsp   1 --------mniivkiqqnlkdevtqlndliisclksdaeliekvgkylve
Ecodsp   1 ------mnlekineltaq---dmagvnaaileqlnsdvqlinqlgyyivs
Hiodsp   1 mkkqdlmsideiqkladp---dmqkvnqnilaqlnsdvpligqlgfyivq
Gsddsp   1 ----------------------mlacnraiiarmespvplipqlgahlva
Rcsdsp   1 ------maidfkqdilapvaqdfaamdqfinegisskvalvmsvskhvve

Rpodsp  43 aggkrirpllltiitakmfdykgn----nhik-lasavefihaatllhddv
Ecoppp  42 gggkrirpmiavlaaravgyegna----hvt-iaaliefihtatllhddv
Hiods  142 gggkrirpliavlaarslgfegsn----sit-catfvefihtasllhddv
Gsddsp  29 aggkrlrpllltlasarlcgyqpgpdhqrhvg-laacvefihtatllhddv
Rcsdsp  45 aggkrmrpimcllaayac----getnlkhaqklaaiiemlhtatlvhddv

Rpodsp  88 vdnstlrrfkptanviwgsktsilvgdflfsqsfklmvasgcikamnvla
Ecoppp  87 vdesdmrrgkatanaafgnaasvlvgdfiytrafqmmtslgslkvlevms
Hiods  277 vdesdmrrgratanaefgnaasvlvgdfiytrafqlvaqleslkilsima
Gsddsp  78 vdestlrrglasanavfgnkasvlvgdflfarsfqlmtadgslkvmails
Rcsdsp  91 vdesglrrgrptanatwnnqtavlvgdfliarafdllvdldnmillkdfs

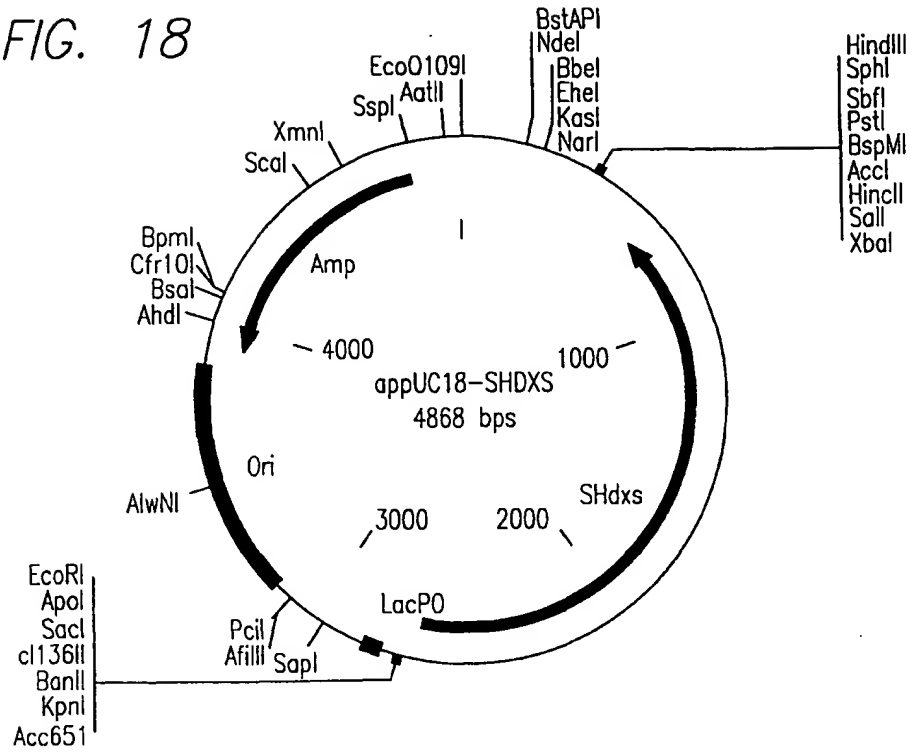
Rpodsp 138 kasviisegevvqlvklnerriitideyqqivksktaelfgaacevgaii
Ecoppp 137 eavnviaegevlqlmnvndpdi-teenymrviysktarlfeaaaqcsgil
Hiods  427 datnvlaegevqqlmnvndpet-seanymrviysktarlfevagqaaaiv
Gsddsp 128 dasatiaegevlqmvvqndltt-pverylevihgktaalfaaacrvgavv
Rcsdsp 141 tgtceiaegevlqlqaqhqpdt-tediylqiihgktsrlfelategaail

Rpodsp 188 aeqvdrvskdvqnfgrllgtifqviddlldylgsdkqvgknigddflegk
Ecoppp 186 agctpeeekglqdygrylgtafqliddlldynadgeqlgknvgddlnegk
Hiods  574 aggteaqekalqdygrylgtafqlvddvldysantqalgknvgddlaegk
Gsddsp 177 aerpeaeeealerfgtnlgmafqlvddaldyaadqqvlgktvgddmregk
Rcsdsp 190 agkpeyre-plrrfaghfgnafqiiddildytsdadtlgknigddlmegk

Rpodsp 238 vtlpliflyhkleqdkqlwlenmlksd--krtkddfvkirdlmlkhaiyn
Ecoppp 236 ptlpllhamhhgtpeqaqmirtaieqgngrhllepvleamnac---gsle
Hiods  724 ptlpllhamrhgnaqqaalireaieqggkreaidevlaimteh---ksld
Gsddsp 227 itlpvlaayeagspedrifwervi--gegeqteddlphalnliaktgain
Rcsdsp 239 ptlpliaamqntqgeqrdlirrsiatggtsqleqviaivqns----gald

Rpodsp 286 etvnylssleneannllnkipv--qniykyylfsiirfilyrsy-
Ecoppp 283 wt---rqraeeeadkaiaalqvlpdtpwrealiglahiavqrdr-
Hiods  865 ya---mnrakeeaqkavdaieilpeseykqalislaylsvdrny*
Gsddsp 275 tt---iaraqvyadaavealsifpdselrrllietvqftvnrar-
Rcsdsp 285 yc---hkrateeteralqaleilpestyrqalvnltrlaldriq-
```

FIG. 18


BstAPI
NdeI
BbeI
EheI
KasI
NarI
HindIII
SphI
SbfI
PstI
BspMI
AccI
HincII
SalI
XbaI
EcoO109I
AatII
SspI
XmnI
ScaI
BpmI
Cfr10I
BsaI
AhdI
Amp
I
4000
1000
appUC18-SHDXS
4868 bps
Ori
AlwNI
3000
2000
SHdxs
LacPO
EcoRI
ApoI
SacI
cl136II
BanII
KpnI
Acc651
PciI
AflIII
SapI

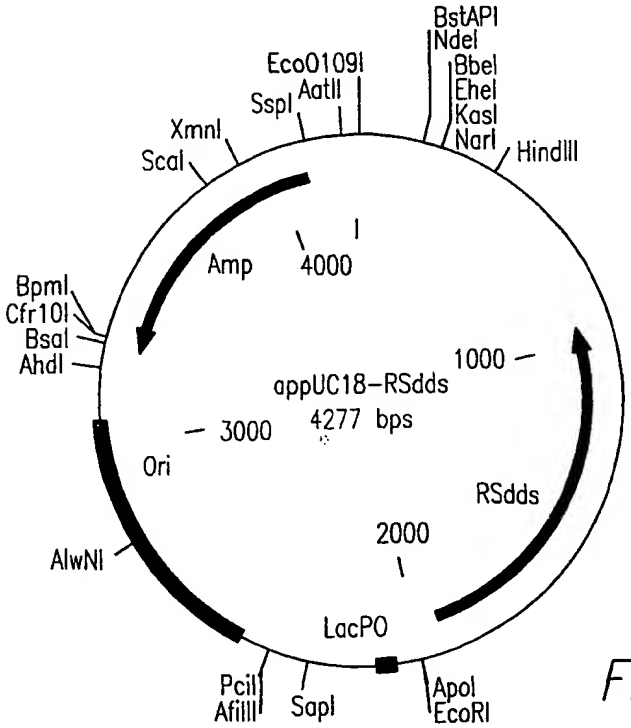
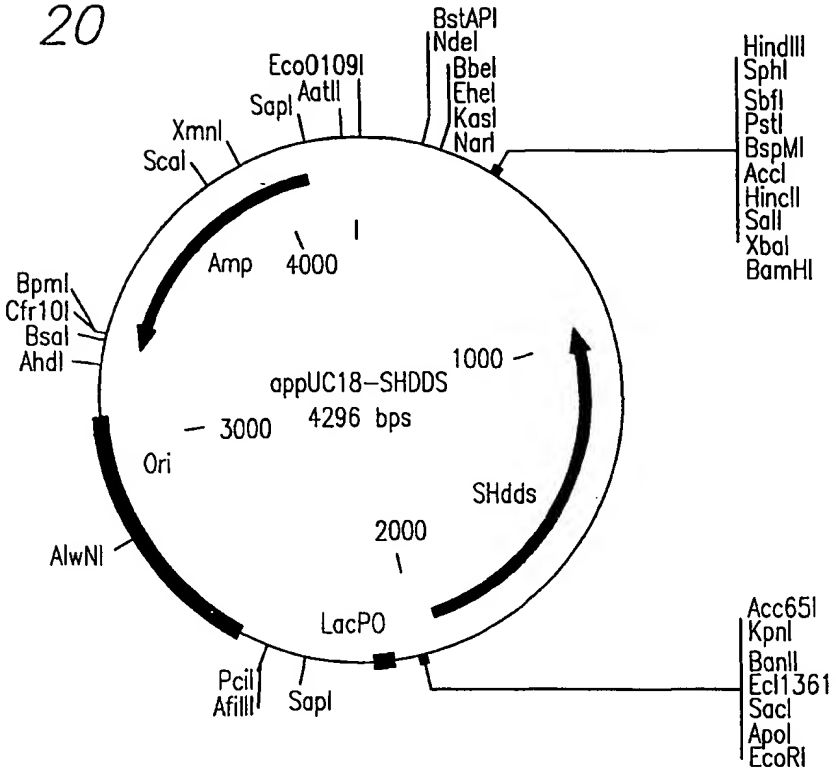

BstAPI
NdeI
BbeI
EheI
KasI
NarI
HindIII
EcoO109I
AatII
SspI
XmnI
ScaI
Amp
4000
I
BpmI
Cfr10I
BsaI
AhdI
1000
appUC18-RSdds
4277 bps
3000
Ori
RSdds
2000
AlwNI
LacPO
PciI
AflIII
SapI
ApoI
EcoRI

FIG. 19

FIG. 20


BstAPI
NdeI
BbeI
EheI
KasI
NarI
Eco0109I
AatII
SapI
XmnI
ScaI
HindIII
SphI
SbfI
PstI
BspMI
AccI
HincII
SalI
XbaI
BamHI
Amp
4000
BpmI
Cfr10I
BsaI
AhdI
1000
appUC18-SHDDS
4296 bps
3000
Ori
SHdds
2000
AlwNI
LacPO
Acc65I
KpnI
BanII
Ecl1361
SacI
ApoI
EcoRI
PciI
AflIII
SapI

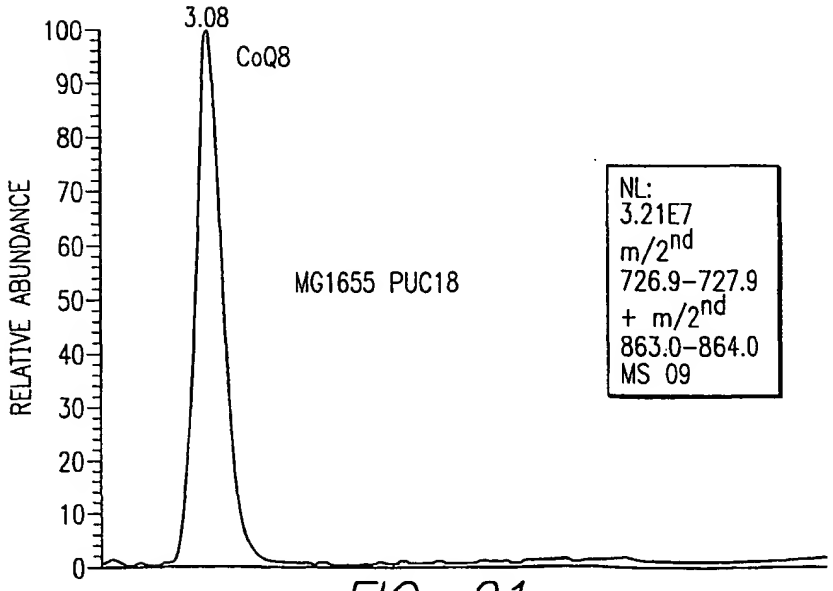

3.08
CoQ8
100
90
80
70
60
50
40
30
20
10
0
RELATIVE ABUNDANCE
MG1655 PUC18
NL:
3.21E7
m/2nd
726.9-727.9
+ m/2nd
863.0-864.0
MS 09

FIG. 21

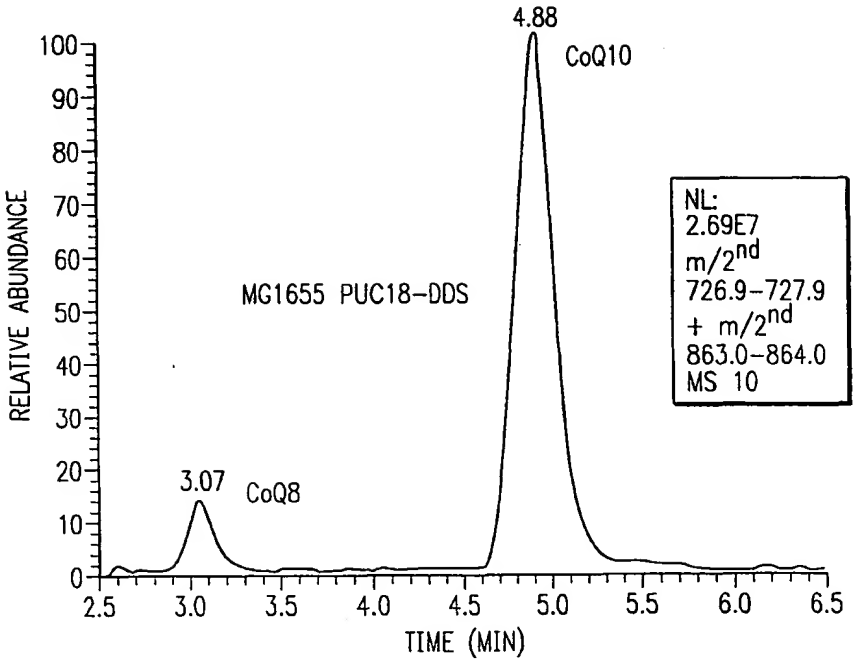

4.88
CoQ10
MG1655 PUC18-DDS
NL:
2.69E7
m/2nd
726.9-727.9
+ m/2nd
863.0-864.0
MS 10
3.07
CoQ8
RELATIVE ABUNDANCE
TIME (MIN)

FIG. 22

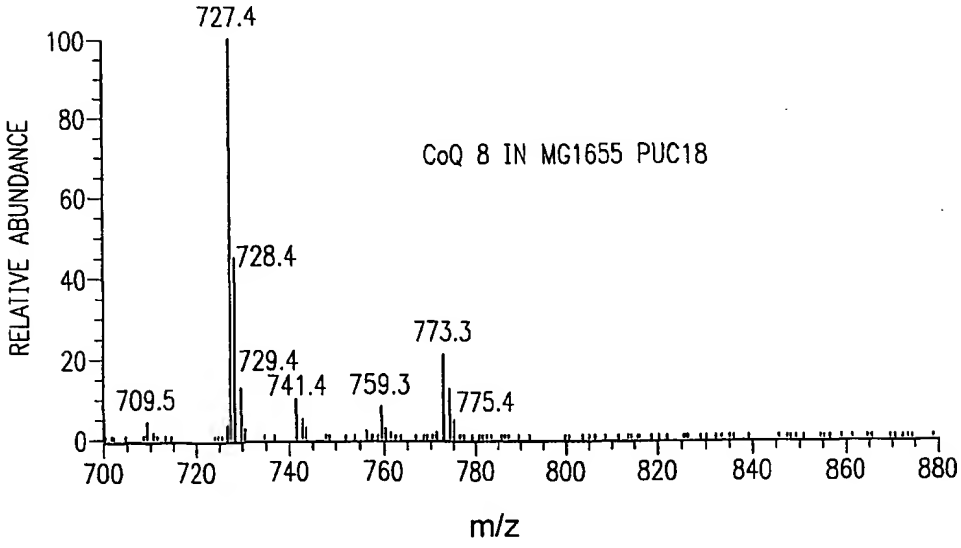

727.4
CoQ 8 IN MG1655 PUC18
728.4
773.3
729.4
741.4
759.3
709.5
775.4
RELATIVE ABUNDANCE
m/z

FIG. 23

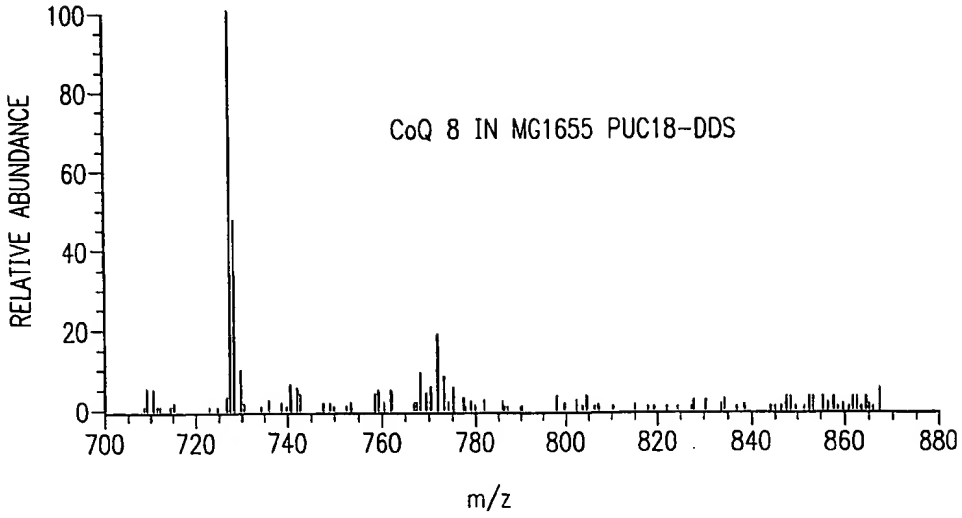

CoQ 8 IN MG1655 PUC18-DDS
RELATIVE ABUNDANCE
100
80
60
40
20
0
700
720
740
760
780
800
820
840
860
880
m/z

FIG. 24

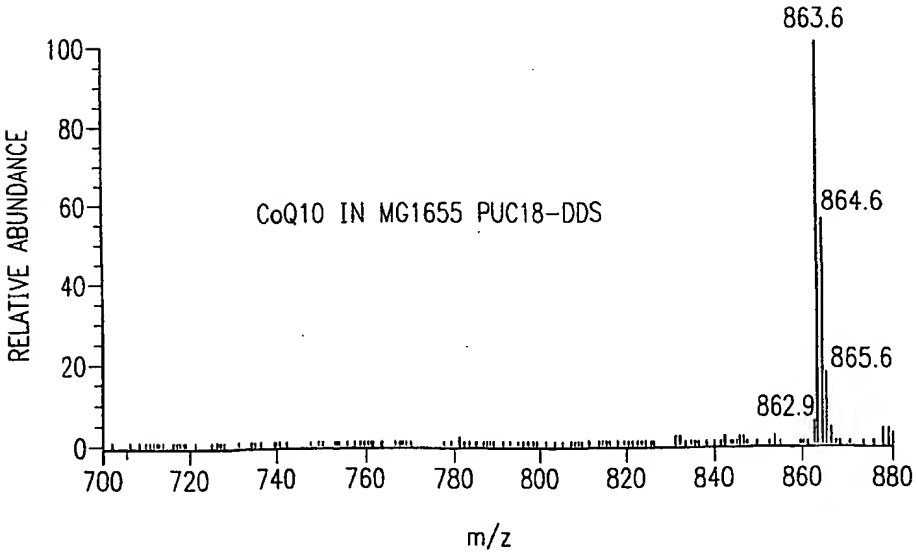

CoQ10 IN MG1655 PUC18-DDS
RELATIVE ABUNDANCE
863.6
864.6
865.6
862.9
100
80
60
40
20
0
700
720
740
760
780
800
820
840
860
880
m/z

FIG. 25

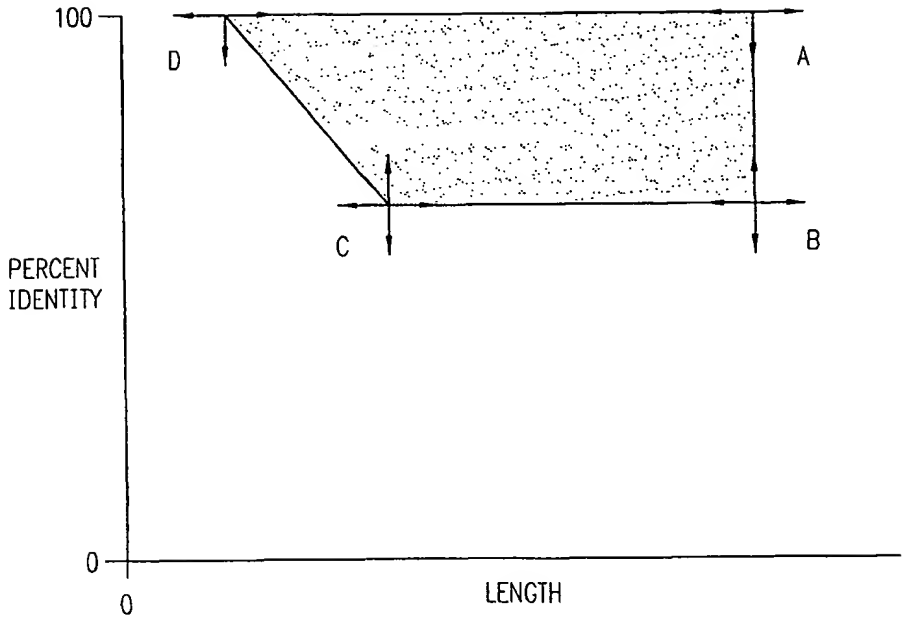


FIG. 26

Figure 27 (page 1 of 2)

```
Bsdxrp   1 ----------------------------------mknicllgatgsigeq
Hmdxrp   1 ---------------------------------mqkqnivilgstgsigks
Ecdxrp   1 ----------------------------------mkqltilgstgsigcs
Zmdxrp   1 ---------------------------------msqprtvtvlgatgsighs
Sldxrp   1 ----------------------------------mkavtllgstgsigtq
Ssdxrp   1 ----------------------------------mvkrisilgstgsigtq
Mtdxrp   1 matggrvvirrrgdnevvahndevtnstdgradgrlrvvvlgstgsigtq

Bsdxrp  17 tldvlrahqdqfqlvsmsfg-rnidkavpmievfqpkfvsvgdldtyhkl
Hmdxrp  19 tlsviennpqkyhafalvgg-knveamfeqcikfrphfaalddvnaakil
Ecdxrp  17 tldvvrhnpehfrvvalvag-knvtrmveqclefspryavmddeasakll
Zmdxrp  20 tldliernldryqvialtan-rnvkdladaakrtnakraviadpslyndl
Sldxrp  17 tldileqypdrfrlvglaag-rnvallseqirrhrpeivaiqdaaqlsel
Ssdxrp  18 tldivthhpdafqvvglaag-gnvallaqqvaefrpeivairqaekledl
Mtdxrp  51 alqviadnpdrfevvglaaggahldtllrqraqtgvtniavadehaaq--

Bsdxrp  66 kqmsfsfec---qiglgeeglieaavmeevdivvnallgsvgliptlkai
Hmdxrp  68 rekli-ahhiptevlagrraicelaahpdadqimasivgaagllptlsav
Ecdxrp  66 ktmlq-qqgsrtevlsgqqaacdmaaledvdqvmaaivgaagllptlaai
Zmdxrp  69 keala---gssveaaagadalve-aammgadwtmaaiigcaglkatlaai
Sldxrp  66 qaaiadl-dnppliltgeagvtevarygdaeivvtgivgcagllptiaai
Ssdxrp  67 kaavaeltdyqpmyvvgeegvvevarygdaesvvtgivgcagllptmaai
Mtdxrp  99 -----rvgdip---yhgsdaatrlveqteadvvlnalvgalglrptlaal

Bsdxrp 113 eqkktialanketlvtaghivkehakkydvpllpvdsehsaifqalqg--
Hmdxrp 117 kagkrvllankeslvtcgqlfidavknygskllpvdsehnaifq---s-l
Ecdxrp 115 ragktillankeslvtcgrlfmdavkqskaqllpvdsehnaifq---s-l
Zmdxrp 115 rkgktvalankeslvsagglmidavrehgttllpvdsehnaifq---c-f
Sldxrp 115 eagkdialanketliaagpvvlpllqkhgvtitpadsehsaifqciqg-l
Ssdxrp 117 aagkdialanketliagapvvlplvekmgvkllpadsehsaifqclqg-v
Mtdxrp 141 ktgarlalankeslvaggslvlraarpg--qivpvdsehsalaqclrggt

Bsdxrp 161 -eqak-----------nierliitasggsfrdktreelesvtvedalkh
Hmdxrp 163 ppeaqekigfcplsel-gvskiiltgsggpfrytpleqftnitpeqavah
Ecdxrp 161 pqpiqhnlgyadleqn-gvvsilltgsggpfretplrdlatmtpdqacrh
Zmdxrp 161 phhnrdy----------vrriiitasggpfrttslaematvtperavqh
Sldxrp 164 sthad----frpaqvvaglrrilltasggafrdwpverlsqvtvadalkh
Ssdxrp 166 pe-------------gglrriiltasggafrdlpverlpfvtvqdalkh
Mtdxrp 189 pde--------------vaklvltasggpfrgwsaadlehvtpeqagah
```

Figure 27 (page 2 of 2)

```
Bsdxrp 198 pnwsmgakitidsatmmnkglevieahwlfdipyeqidvvlhkesiihsm
Hmdxrp 212 pnwsmgkkisvdsatmmnkgleyiearwlfnasaeemeviihpqsiihsm
Ecdxrp 210 pnwsmgrkisvdsatmmnkgleyiearwlfnasasqmevlihpqsvihsm
Zmdxrp 200 pnwsmgakisidsatmmnkglelieayhlfqiplekfeilvhpqsvihsm
Sldxrp 210 pnwsmgrkitvdsatlmnkglevieahylfgldydyidivihpqsiihsl
Ssdxrp 202 pnwsmgqkitidsatlmnkglevieahylfgldydhidivihpqsiihsl
Mtdxrp 224 ptwsmgpmntlnsaslvnkglevieth1lfgipydridvvvhpqsiihsm

Bsdxrp 248 vefhdksviaqlgtpdmrvpiqyaltypdrlplpdakrlelweigslhfe
Hmdxrp 262 vryvdgsvitqmgnpdmrtpiaetmayphrtfa-gvepldffkikeltfi
Ecdxrp 260 vryqdgsvlaqlgepdmrtpiahtmawpnrvns-gvkpldfcklsaltfa
Zmdxrp 250 veyldgsilaqigspdmrtpightlawpkrmet-paesldftklrqmdfe
Sldxrp 260 ieledtsvlaqlgwpdmrlpllyalswpdrlst-qwsaldlvkagslefr
Ssdxrp 252 ievqdtsvlaqlgwpdmrlpllyalswperiyt-dwepldlvkagslsfr
Mtdxrp 274 vtfidgstiaqasppdmklpislalgwprrv-sgaaaacdfhtasswefe

Bsdxrp 298 kadfdrfrclqfafesgkiggtmptvlnaanevavaaflagkipflaied
Hmdxrp 311 epdfnrypnlklaidafaagqyattamnaaneiavqafldrqigfmdiak
Ecdxrp 309 apdydrypclklameafeqgqaattalnaaneitvaaflaqqirftdiaa
Zmdxrp 299 apdyerfpaltlamesiksggarpavmnaaneiavaafldkkigfldiak
Sldxrp 309 epdhakypcmdlayaagrkggtmpavlnaaneqavalfleeqihfsdipr
Ssdxrp 301 epdhdkypcmqlaygagraggampavlnaaneqavalflqekisfldipr
Mtdxrp 323 pldtdvfpavelarqagvaggcmtavynaaneeaaaaflagrigfpaivg

Bsdxrp 348 cieka--ltrhqllkkpswr---tfkkwtk---------ipgdtsiqysh
Hmdxrp 361 inskt--ierispytiqniddvleidaqare--------ia-ktllre--
Ecdxrp 359 lnlsv--lekmdmrepqcvddvlsvdanare--------varkevmrlas
Zmdxrp 349 ivekt--ldhytpatpssledvfaidnear---------iqaaalmeslp
Sldxrp 359 lieracdrhqtewqqqpslddilaydawarqfv------qasyqslesvv
Ssdxrp 351 liektcdlyvgqntaspdletilaadqwarrtv------lensacvatrp
Mtdxrp 373 iiadvlhaadqwavepatvddvldaqrwareraqravsgmasvaiastak

Bsdxrp 384 kvvcs---------
Hmdxrp 398 ----------
Ecdxrp 399 ----------
Zmdxrp 388 a----------
Sldxrp 403 ----------
Ssdxrp 395 ----------
Mtdxrp 423 pgaagrhastlers
```

Figure 28

ggcccgggctggtggggtttctggcgctggggctggtgttcggcgcgttcttcttcgtcg
cgatcgtgacgcggaacgccaagctggcggcggggcaggtctatgtcgggctgccggtgc
tcgcgctgctgctgctccgcgaccatccgcagggctttgccgcgacgctgtggacgatgg
cgatcgtctgggtgtgcgacagcggcgcctattttgccggtcgcgcgatcggtgggccca
agctcgcgccctcgatcagcccgaacaagacctgggcggggctgatcggcgggttggttg
ccgcgatcctgttctccgccggctatgtcgcgctggcgccggggagcgcgatcggctggt
ggctggtcgcggtgtcgccgctggtagccttcgcctcgcagatcggcgacctgtacgaga
gccatctcaagcgggtcgcgggcgtgaaggattcgagcaacctgctgcccggccatggcg
gcattctcgaccggctcgacggccttgtcttcgcagccccggttgcagctttgtttttg
cgatccatcatcaggtggtcgtgggaggatactggtggtgaagcgcgtcacggtgttggg
ggcgaccggctcggtcggcacctcgacgctggatctgatcgaacgaaatccgcacgcctt
cgaagtcgtggcgctgaccgcaaattgcgatgtcgagaagctggctgccgcggcgatccg
cacgcgcgcgcgctgcgccgtggtcgccgacgagaaatgcctgccggcgctacaggagcg
gctggccggcagcggtgtcgaggcgatgggcggggcgcattcggtgtgcgacgtggcgcg
gatgggtgctgactggacgatggctgcgatcgtcggcagcgcagggctcaagccggtgat
ggccgcgctggaggccggtggcaccgtcgcgctcgcgaacaaggagtcgctcgtctcggc
gggtgaggtgatgatggcggcggcccgcgcgcatggcgcgacgctgctgccggtcgattc
ggagcacaatgcggtgttccagtgcctcgatcgcaccgcgcccaggggcgtccgccggat
catccttaccgccagcggtggtccgttccgcgcgacgccgaaggaagcgatgcgcgacat
caccccccgcacaggcggtggcgcatcccaactggtcgatgggcgccaagatctcggtcga
ctccgcgacgatgatgaacaaggggctcgaactgatcgaagccttccacctgttcccggt
cgccgccgagcaactggccgtgctggtccatcgccaatccgtcgtccattcgatggtgga
atatgtcgacggatcggtgctggcccagctcggcacgcccgacatgcgcacgccgatcgc
ctatgcgctggcttggcccgagcggatggagacgctgtgcccgccgctcgaccttgccac
ggtgggtaagctcgagttcgaaaatcccgatctcgatcgcttcccggcgctcgcgctggc
gatggaggcattgaaggcgggcggggcgcgtccggccattctcaatgccgccaacgaagt
cgccgtcgcggcctttctcgccgggcggatcggattccttgaaattgccgcaatctctgc
cgatacgctgtctcgctatgacccggccgcgccggaaacgctcgatgccgtgctggcgat
cgacgcggaggcgcggctttacgcggctgagcgagtgaaggactgcgtcgcttgatccaa
tcccccggcatcctgctcaccattctggcgttcgcgctggtgatcgggccgctcgtgttc
ctgcacgagctgggacattatctggcgggccgcctcttcggggtgaaggccgaggaattc
tcgatcggcttcggccgcgagatcgccggcaccaccgatcgccgcggcacgcgctggaag
ttcagcctgttgccgctgggcggctatgtccgcttcgccggcgacatgaacccggcgagc
cagccttcgcccgaatggctgcagaccagcccgggcc (SEQ ID NO:95)

Figure 29

gtggtgaagcgcgtcacggtgttgggggcgaccggctcggtcggcacctcgacgctggat
ctgatcgaacgaaatccgcacgccttcgaagtcgtggcgctgaccgcaaattgcgatgtc
gagaagctggctgccgcggcgatccgcacgcgcgcgctgcgccgtggtcgccgacgag
aaatgcctgccggcgctacaggagcggctggccggcagcggtgtcgaggcgatgggcggg
gcgcattcggtgtgcgacgtggcgcggatgggtgctgactggacgatggctgcgatcgtc
ggcagcgcagggctcaagccggtgatggccgcgctggaggccggtggcaccgtcgcgctc
gcgaacaaggagtcgctcgtctcggcgggtgaggtgatgatggcggcggcccgcgcgcat
ggcgcgacgctgctgccggtcgattcggagcacaatgcggtgttccagtgcctcgatcgc
accgcgcccaggggcgtccgccggatcatccttaccgccagcggtggtccgttccgcgcg
acgccgaaggaagcgatgcgcgacatcacccccgcacaggcggtggcgcatcccaactgg
tcgatgggcgccaagatctcggtcgactccgcgacgatgatgaacaaggggctcgaactg
atcgaagccttccacctgttcccggtcgccgccgagcaactggccgtgctggtccatcgc
caatccgtcgtccattcgatggtggaatatgtcgacggatcggtgctggcccagctcggc
acgcccgacatgcgcacgccgatcgcctatgcgctggcttggcccgagcggatggagacg
ctgtgcccgccgctcgaccttgccacggtgggtaagctcgagttcgaaaatcccgatctc
gatcgcttcccggcgctcgcgctggcgatggaggcattgaaggcgggcggggcgcgtccg
gccattctcaatgccgccaacgaagtcgccgtcgcggcctttctcgccgggcggatcgga
ttccttgaaattgccgcaatctctgccgatacgctgtctcgctatgacccggccgcgccg
gaaacgctcgatgccgtgctggcgatcgacgcggaggcgcggctttacgcggctgagcga
gtgaaggactgcgtcgcttga (SEQ ID NO:96)

Figure 30

```
  1  vvkrvtvlga tgsvgtstld liernphafe vvaltancdv eklaaaairt
 51  rarcavvade kclpalqerl agsgveamgg ahsvcdvarm gadwtmaaiv
101  gsaglkpvma aleaggtval ankeslvsag evmmaaarah gatllpvdse
151  hnavfqcldr taprgvrrii ltasggpfra tpkeamrdit paqavahpnw
201  smgakisvds atmmnkglel ieafhlfpva aeqlavlvhr qsvvhsmvey
251  vdgsvlaqlg tpdmrtpiay alawpermet lcppldlatv gklefenpdl
301  drfpalalam ealkaggarp ailnaaneva vaaflagrig fleiaaisad
351  tlsrydpaap etldavlaid aearlyaaer vkdcva  (SEQ ID NO:97)
```

Figure 31 (page 1 of 15)

```
Stdxrcds     1 --------------------------------------------------
Padxrd       1 at------------------------------------------------
Zmdxrd       1 --------------------------------------------------
Sgdxrd       1 --------------------------------------------------
Nmdxrd       1 --------------------------------------------------
Ecdxrd       1 --------------------------------------------------
Sldxrd       1 --------------------------------------------------
Mldxrd       1 --------------------------------------------------
Pmdxrp       1 atgagtattagttat------------------------------------
Atdxrd       1 atgatgacattaaactcactatctccagctgaatccaaagctatttcttt
Cjdxrd       1 --------------------------------------------------
Pfdxrd       1 --------------------------------------------------

Stdxrcds     1 ------------------------------------------gtgg-----
Padxrd       3 ------------------------------------------gagt-----
Zmdxrd       1 ------------------------------------------atga-----
Sgdxrd       1 ------------------------------------------ttgg-----
Nmdxrd       1 ---------------------------------------------a-----
Ecdxrd       1 ---------------------------------------------a-----
Sldxrd       1 ---------------------------------------------g-----
Mldxrd       1 ---------------------------------------------g-----
Pmdxrp      16 ------------------------------------------ttta-----
Atdxrd      51 cttggatacctccaggttcaatccaatccctaaactctcaggtgggttta
Cjdxrd       1 --------------------------------------------------
Pfdxrd       1 ---------------------------------------------a-----

Stdxrcds     5 --------------------------------------------------
Padxrd       7 --------------------------------------------------
Zmdxrd       5 --------------------------------------------------
Sgdxrd       5 --------------------------------------------------
Nmdxrd       2 --------------------------------------------------
Ecdxrd       2 --------------------------------------------------
Sldxrd       2 --------------------------------------------------
Mldxrd       2 --------------------------------------------------
Pmdxrp      20 --------------------------------------------------
Atdxrd     101 gtttgaggaggaggaatcaagggagaggttttggaaaaggtgttaagtgt
Cjdxrd       1 --------------------------------------------------
Pfdxrd       2 --------------------------------------------------
```

Figure 31 (page 2 of 15)

```
Stdxrcds     5 ----------------------------------------------------
Padxrd       7 ----------------------------------------------------
Zmdxrd       5 ----------------------------------------------------
Sgdxrd       5 ----------------------------------------------------
Nmdxrd       2 ----------------------------------------------------
Ecdxrd       2 ----------------------------------------------------
Sldxrd       2 ----------------------------------------------------
Mldxrd       2 ----------------------------------------------------
Pmdxrp      20 ----------------------------------------------------
Atdxrd     151 tcagtgaaagtgcagcagcaacaacaacctcctccagcatggcctgggag
Cjdxrd       1 ----------------------------------------------------
Pfdxrd       2 ----------------------------------------------------

Stdxrcds     5 ---------tga----ag-------------------------------
Padxrd       7 ---------cgaccgcag-------------------------------
Zmdxrd       5 ---------gtc----ag-------------------------------
Sgdxrd       5 ---------tca--------------------------------------
Nmdxrd       2 ---------tga----ca-------------------------------
Ecdxrd       2 ---------tga----ag-------------------------------
Sldxrd       2 ---------tga----aa-------------------------------
Mldxrd       2 ---------tga----acaatccgatcgaggggcacgctggcggccgcct
Pmdxrp      20 ---------tga----aa-------------------------------
Atdxrd     201 agctgtccctga----gg-------------------------------
Cjdxrd       1 ----------------------------------------------------
Pfdxrd       2 ---------tga----ag-------------------------------

Stdxrcds    10 -cg------c------gtca-cggtgttgggggcgacc------------
Padxrd      16 -cg------g------atca-gcgtgctcggcgcgacc------------
Zmdxrd      10 -cc------aagaacagtca-ctgttttaggggcgacc------------
Sgdxrd       8 ------------------------ttctcggctcgacc------------
Nmdxrd       7 -ccacaagtc------ctga-ccatattaggcagtacc------------
Ecdxrd       7 -ca------a------ctca-ccattctgggctcgacc------------
Sldxrd       7 -gc------a------gtga-cactgctcggttcaacc------------
Mldxrd      39 ccg------c------gtgc-tggtgttgggaagtact------------
Pmdxrp      25 -aa------g------atcg-ttattttaggttcaact------------
Atdxrd     215 -cg------c------ctcgtcaatcttgggatggaccaaaacccatctc
Cjdxrd       1 ----------------atga-tacttttggaagtacg------------
Pfdxrd       7 ------------------aa-atatatttatatatatt------------
```

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```
Stdxrcds    34 ----------------ggctcggtcggcacctcgacgctggatc------
Padxrd      40 ----------------ggctcgatcggcctgagcaccctggacg------
Zmdxrd      40 ----------------ggatccattggtcattcaacactggatt------
Sgdxrd      22 ----------------ggctcgatcggcacccaggccatcgacg------
Nmdxrd      37 ----------------ggcagcataggcgaaagcacgctggacg------
Ecdxrd      31 ----------------ggctcgattggttgcagcacgctggacg------
Sldxrd      31 ----------------ggctcgatcgggacacaaaccctagaca------
Mldxrd      64 ----------------ggctcaattggcacccaggcgctggaag------
Pmdxrp      49 ----------------ggatcgattggtaccagtactttatccg------
Atdxrd     252 tatcgttggatctactggttctattggcactcagacattggata------
Cjdxrd      22 ----------------ggc---------------agtataggag------
Pfdxrd      26 ----------------ttttct-tcatcacaataactattaatgatttag

Stdxrcds    62 -------tgatcgaacgaaatccgcacgccttcgaagtcg------tggc
Padxrd      68 -------tcgtccagcgtcatcccgatcgttacgaagcct------tcgc
Zmdxrd      68 -------taatcgaacggaatttagatcggtatcaggtca------tcgc
Sgdxrd      50 -------tggtgctccgcaaccccggccggttcaaggtgg------tcgc
Nmdxrd      65 -------ttgtctcccgccaccccgaaaaattccgcgtat------tcgc
Ecdxrd      59 -------tggtgcgccataatcccgaacacttccgcgtag------ttgc
Sldxrd      59 -------ttcttgagcagtatcccgatcgctttcgcctcg------tagg
Mldxrd      92 -------ttatcgccgccaatccggaccgtttcgaggtag------tcgg
Pmdxrp      77 -------tgattacacataatcctgataagtaccaagtgt------ttgc
Atdxrd     296 -------ttgtggctgagaatcctgacaaattcagagttg------tggc
Cjdxrd      35 -------taaatgctcttaaacttgctgctttaaaaaaca------ttcc
Pfdxrd      59 taataaataatacatcaaaatgtgtttccattgaaagaagaaaaaataac

Stdxrcds    99 gct----------------------------gaccgca------aattgc
Padxrd     105 cct----------------------------gactggc------ttcagc
Zmdxrd     105 ttt----------------------------gaccgcc------aaccgc
Sgdxrd      87 gct----------------------------gtccgcg------gccggc
Nmdxrd     102 gct----------------------------ggcaggg------cataag
Ecdxrd      96 gct----------------------------ggtggca------ggcaaa
Sldxrd      96 gct----------------------------ggcggct------ggtcgt
Mldxrd     129 gct----------------------------ggccgc------------c
Pmdxrp     114 gtt----------------------------agttggt------ggacgt
Atdxrd     333 tct----------------------------agctgct------ggttcg
Cjdxrd      72 cat----------------------------ttctgct------ttagct
Pfdxrd     109 gcatatataaattatggtataggatataatggaccagataataaaataac
```

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```
Stdxrcds  115 ---------gatgtcgag--aagctgg-----c----------------tgc
Padxrd    121 cgcctggccgaactcgag--gcgctg----------------------tgc
Zmdxrd    121 ---------aatgtcaaa--gatctgg-----c----------------cga
Sgdxrd    103 ---------ggcgcggtg--gagctgc-----t----------------cgc
Nmdxrd    118 ---------caggtcgag--aaattgg-----c----------------ggc
Ecdxrd    112 ---------aatgtc-ac--tcgcatg-----g----------------tag
Sldxrd    112 ---------aatgtggcg--ctgtt-------------------------
Mldxrd    139 ---------gggggcgcg--cagctggacacgc----------------tgc
Pmdxrp    130 ---------aatgtagagctaatgttt-----c----------------aac
Atdxrd    349 ---------aatgttact--ctacttg-----c----------------t--
Cjdxrd     88 ---------tgtggggat--aacatcg-----c----------------t--
Pfdxrd    159 ---------aaagagtag--aagatgt-----aaaagaataaagttatgc

Stdxrcds  135 -cgcg---gcgatc--cgcac-g-cgcgcgc-gctgc--g-c-------c
Padxrd    148 -ctca---ggcacc--gcccc-g-tctatgc-ggtggt-g-c-------c
Zmdxrd    141 -tgcg---gcgaaa--agaac-g-aatgcca-agcgg--g-c-------g
Sgdxrd    123 -cgag---caggccgtcgcactg-ggcgtgc-acacc--g-t-------c
Nmdxrd    138 tcaat---gtcaaa--cgttc---caccccg-aatat--g-c-------c
Ecdxrd    131 -aaca---gtgcct--ggaat-t-ctctccccgctat--g-c-------c
Sldxrd    126 ----g---tcggag--caaat-t-cggcggc-accga--c-c-------a
Mldxrd    164 -tgag---gc----------------agcgc-gccgc--gac-------c
Pmdxrp    152 -aatgtttgacatt--ccaac-c-gtcgttt-gctgc--g-ttagatgac
Atdxrd    367 ----------gatc--aggta-a-ggagatt-taagcctg-c-------a
Cjdxrd    106 -cttt---taaatg--agcaa-atcgcaagg-tttaa--a-c-------c
Pfdxrd    193 -aaaa---aggat----ttaa-t-agatatt-ggtgc--a-a-------t

Stdxrcds  166 gtggtcgc--cg------ac---------ga---------gaaatgc---
Padxrd    180 ggagcagg--cc------gc---------gg---------cgattgc---
Zmdxrd    172 gttatcgc--tg------ac---------cc---------gtcgctt---
Sgdxrd    157 gcggtggc--cg------acccggccgccga---------ggaagccg--
Nmdxrd    169 gtcgttgc--cg------at---------gc---------cgaa--c---
Ecdxrd    163 gtaatgga--cg------at---------gaagcgagtgcgaaactt---
Sldxrd    154 gagattgtggcg------at---------tc---------aagatgcagc
Mldxrd    184 ggcgtcac--ca------at---------atc--------gccatcg---
Pmdxrp    193 gatgtcgc--ag------cc-------------------aaaatgt---
Atdxrd    394 ttggttgc--tgttagaaac---------ga---------gtcactg---
Cjdxrd    138 caaatttg--tt------tc---------ca---------taaaaga---
Pfdxrd    222 aaagaaac--ca------at---------taatgta----gcaattt---
```

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```
Stdxrcds  187 ----ctg------c---cg---gc--gctacagg------agcggctg--
Padxrd    201 ----ctt------g---ca---gg--gct-cgct------cgccgc-g--
Zmdxrd    193 ----tat------a---at---ga--tctgaaag------aggctttg--
Sgdxrd    188 ----ctg------c---gc---ga--ggccctggcggccaaggcgcag--
Nmdxrd    188 ----acg------c---cg---cccggcttgaag------ccctgttgaa
Ecdxrd    193 ----ctt------aaaacg---at--gctacagc------aacag-----
Sldxrd    180 tcagctg------t---cg---ga--actgcaag------cggcgatc--
Mldxrd    206 ----ctg------a---cgatcgc--gc----gg------ctcagctg--
Pmdxrp    212 ----tgg------c---------------agaga------aactgaaa--
Atdxrd    421 ----att------a---at---ga--gcttaaag------aggcttta--
Cjdxrd    159 ----tt-----------------c--aaaaaata------agcattta--
Pfdxrd    248 ----ttggaagtac---tg---gt--agtatagg------tacgaatg--

Stdxrcds  211 ----gcc----------ggcagcgg----------
Padxrd    223 ----gcg----------ggtatccg----------
Zmdxrd    217 ----gcc----------ggaagctc----------
Sgdxrd    218 ----ggc----------gcccgctg----------
Nmdxrd    216 acgcgac----------ggca-cgg----------
Ecdxrd    217 ----------------ggtagccg----------
Sldxrd    208 ----gca----------gaccttga----------
Mldxrd    229 ----gcc----------ggc----------
Pmdxrp    229 ----gcc----------caccaa----------
Atdxrd    445 ----gct----------gatttgga----------
Cjdxrd    178 ----gtt----------aaacacga----------
Pfdxrd    278 ----ctttaaatataataagggagtgtaataaaattgaaaatgtttttaa

Stdxrcds  222 tg------tcg-ag-----gcgat-gggcggggc----------gca
Padxrd    234 ca------ccc-gg-----gtgct-gttcggcga----------gca
Zmdxrd    228 tg------ttg-ag-----gcagc-cgcgggtgc----------tga
Sgdxrd    229 cc------gcg--g-----gtgct-ggcgggccc----------gga
Nmdxrd    230 cg------actcag-----gtttt-acacggcgc----------gca
Ecdxrd    225 ca------ccg-aa-----gtctt-aagtgggca----------aca
Sldxrd    219 ta------atc-cg-----ccgct-catcctgac----------
Mldxrd    235 -g------aca-tc-----cctta-ccacgggac----------cga
Pmdxrp    238 ag------cca-aacaacagtctt-agcaggaca----------gca
Atdxrd    456 ctataaactcg-ag-----attat-tccaggaga----------gca
Cjdxrd    189 ta------gag-tt-----tttatagggcaagaa----------ggt
Pfdxrd    324 tg------tta-aa-----gcatt-gtatgtgaataagagtgtgaatgaa
```

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```
Stdxrcds  246 ttcgg------tgtgcgacgtggc----g---------cgga--------
Padxrd    258 ggcgt------tgtgcgaagtggc----c---------ag----------
Zmdxrd    252 tgcct------tggtcgaagccgc----c---------atga--------
Sgdxrd    252 cgcgg------cgaccgagctggcc---g---------cggc--------
Nmdxrd    255 ggcat------tggttgacgttgcctctg---------ccga--------
Ecdxrd    249 agccg------cttgcgatatggca---g---------cgct--------
Sldxrd    240 --cgg------tgaggcaggtgtc----a---------cgga--------
Mldxrd    258 tgcg---------------gtcac----c---------cggc--------
Pmdxrp    267 agcca------tttgtgagttagc----gg--------caca--------
Atdxrd    486 aggag------tgattgaggttgc----c---------cgac--------
Cjdxrd    214 ttagagcaaattttaacagaatgt----c---------aaga--------
Pfdxrd    361 ttata------tgaacaagctaga----gaatttttaccagaatatttgt

Stdxrcds  269 ----------------------------------------------------
Padxrd    279 ----------------------------------------------------
Zmdxrd    275 ----------------------------------------------------
Sgdxrd    276 ----------------------------------------------------
Nmdxrd    282 ----------------------------------------------------
Ecdxrd    273 ----------------------------------------------------
Sldxrd    261 ----------------------------------------------------
Mldxrd    272 ----------------------------------------------------
Pmdxrp    291 ----------------------------------------------------
Atdxrd    509 ----------------------------------------------------
Cjdxrd    243 ----------------------------------------------------
Pfdxrd    401 gtatacatgataaaagtgtatatgaagaattaaaagaactggtaaaaaat

Stdxrcds  269 -------------------------tg----------gg--tgctga--
Padxrd    279 -------------------------cg----------cg--cccgaa--
Zmdxrd    275 -------------------------tg----------gg--tgccga--
Sgdxrd    276 -------------------------gg----------ag--tgcc-a--
Nmdxrd    282 -------------------------cg----------aa--gtcag---
Ecdxrd    273 -------------------------tg----------aggatgttga--
Sldxrd    261 -------------------------agtggctcgctacgg--tgatgc--
Mldxrd    272 -------------------------tg----------gt--tgaggaga
Pmdxrp    291 -------------------------tcct--------ga--agcaga--
Atdxrd    509 -------------------------at----------cc--tgaagc--
Cjdxrd    243 -------------------------ta----------ag--ctttta--
Pfdxrd    451 ataaaagattataaacctataatattg----------tg--tggtga--
```

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```
Stdxrcds  279 ctg-----gacg-----atgg---c-----tgcg---atc----gtcggc
Padxrd    289 gtg-----gacatggtaatgg---c-----ggcc---atc----gtcggc
Zmdxrd    285 ttg-----gaca-----atgg---c-----agcc---att----atcggt
Sgdxrd    285 ctc-----ggtg-----ctga---a-----cggc---atc----accggt
Nmdxrd    291 cgg-----tgtc-----atgt---g-----cgcc---atc----gtcggg
Ecdxrd    285 tca-----ggtg-----atgg---c-----agcc---att----gttggc
Sldxrd    282 cga-----gatt-----gtggtcac-----tggc---att----gtcggt
Mldxrd    284 ctgaggctgacg-----ttgt---cctcaatgcg---ctg----gtcggg
Pmdxrp    303 tat-----ggta-----atgg---c-----tgcg---att----gtgggg
Atdxrd    519 tgt-----aacc-----gttg---t-----taccggaata----gtaggt
Cjdxrd    253 ctc-----aa--------------------tgcc---att----gtaggt
Pfdxrd    486 tga-----aggg-----atga---a-----agaa---atatgtagtagta

Stdxrcds  304 agcgcagggctcaagccggtgatgg-------------------------
Padxrd    319 gccgccgggctgccgtcgaccctgg-------------------------
Zmdxrd    310 tgcgccggtctaaaagcgacgcttg-------------------------
Sgdxrd    310 tcgatcggcctggccccgacgctgg-------------------------
Nmdxrd    316 gcggtggggctgccttccgcgctcg-------------------------
Ecdxrd    310 gctgctgggctgttacctacgcttg-------------------------
Sldxrd    310 tgcgctggtctgctacccacgatcg-------------------------
Mldxrd    319 gcattgggtctgcgacccacactgg-------------------------
Pmdxrp    328 gcggcgggattattgcctactttgt-------------------------
Atdxrd    547 tgtgcgggactaaagcctacggttg-------------------------
Cjdxrd    271 tttgcaggacttaaaagcactttaa-------------------------
Pfdxrd    515 atagtatagataaaatagttattggtattgattcttttcaaggattatat

Stdxrcds  329 -ccgcgctggaggccggtggcacc---------gtcgcgctcgcgaacaa
Padxrd    344 -cggccgtcgaggccggcaagcgc---------gtactgctggccaacaa
Zmdxrd    335 -cagctattcgcaagggcaaaacg---------gtcgctttagcgaataa
Sgdxrd    335 -ccgcgctgcgggccggccgggtg---------ctggtgctggcgaacaa
Nmdxrd    341 -cagcggcgcaaaaaggcaaaacc---------atttatctggcgaacaa
Ecdxrd    335 -ctgcgatccgcgcgggtaaaacc---------attttgctggccaataa
Sldxrd    335 -ccgcgatcgaagccggcaaggat---------atcgcccttgccaacaa
Mldxrd    344 -ctgcactgcacacgggcgcgcga---------ttggcgttggccaacaa
Pmdxrp    353 -ctgcggtgaaagctggaaaacgt---------gtactattagcaaataa
Atdxrd    572 -ctgcaattgaagcaggaaaggac---------attgctcttgcaaacaa
Cjdxrd    296 -aggctaaagagcttggcaaaaac---------atagctttagctaacaa
Pfdxrd    565 tctactatgtatgcaattatgaataataaaatagttgcgttagctaataa
```

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```
Stdxrcds  369 ggagtcgctcgtctcggcgggtgaggtgatgatgg-cggcggcccgc-gc
Padxrd    384 ggaggcgctggtgatgtccggcgcgctgttcatgc-aggcggt-caa-gc
Zmdxrd    375 ggaatccttagtttcagctggcggattgatgatcg-atgccgtgcgg-ga
Sgdxrd    375 ggagtcgctgatcgtcggcggtccgctggtgaagg-cggtg-------gc
Nmdxrd    381 agagacgctggtggtttccggcgcgttgtttatgg-aaaccgcccgt-gc
Ecdxrd    375 agaatcactggttacctgcggacgtctgtttatggacgccgtaaagcaga
Sldxrd    375 agaaaccctgattgcagcaggcccagtggtcctgc-cactcctgcaa-aa
Mldxrd    384 ggaatcgctggtagctggcggttcgctggtgttgg-ccgcggcgc----a
Pmdxrp    393 agaagccttggtaacttgcgggcaattatttattg-atgcagtgcgt-ga
Atdxrd    612 agagacattaatcgcaggtggtcctttcgtgcttc-cgcttgccaac-aa
Cjdxrd    336 agaaagtcttgtagtagctgg-gagtttttt-------------------
Pfdxrd    615 agaatccattgtctctgctggtttctttttaaaga-aattattaaat-at

Stdxrcds  417 gcat-ggc---gcgacgctgctgccggtcgattcggagcacaatgcggtg
Padxrd    431 gcagcggc---gcggtgctcctgccgatcgacagcgagcacaacgcgatc
Zmdxrd    423 acat-ggc---acgacgcttctccccgtcgattccgagcataacgctatt
Sgdxrd    417 gcag-ccc---ggccagatcgtgccggtggactccgagcacgccgcgctg
Nmdxrd    429 aaac-ggc---gcggcagtgctgcccgtcgacagcgaacacaacgccgtt
Ecdxrd    425 gcaa-agc---gcaat--tgttaccggtcgatagcgaacataacgccatt
Sldxrd    423 gcac-ggt---gtcaccattacgcctgccgactccgagcactccgcgatc
Mldxrd    429 gcca-ggc---caga---tcgtgcccgtagactcggaacactccgcgctg
Pmdxrp    441 atct-caa---gcacaattgttaccagtagatagtgaacataatgcgatt
Atdxrd    660 acat-aat---gtaaagattcttccggcagattcagaacattctgccata
Cjdxrd    366 gaaa-ggg---gctaaatttttacccgttgatagtgagc---atgcagct
Pfdxrd    663 tcat-aaaaatgcaaagataatacctgttgattcagaacatagtgctata

Stdxrcds  463 ttccag----t-------gc---ct---------------cg--at----
Padxrd    478 ttccag----t-------cg---ctgccgcgcaattatgccg--at----
Zmdxrd    469 ttccaa----t-------gc---tt---------------c----c----
Sgdxrd    463 ttccag----g-------cg---ct---------------gg--cc----
Nmdxrd    475 ttccaagtttt-------gc---cg---------------cgcgat----
Ecdxrd    469 tttcag----a-------g-----t---------------tt--ac----
Sldxrd    469 tttcag----t-------gc---at---------------cc--aa----
Mldxrd    472 gcgcaa----t-------gc---ctgcg------------cg--gt----
Pmdxrp    487 ttccaa----tcccttccgc---ct---------------ga--ag----
Atdxrd    706 tttcag----t-------gt---at-------------------t----
Cjdxrd    409 ttaaaa----t-------ttttact---------------cg--aa----
Pfdxrd    712 tttcaa----t-------gt---tt---------------ag-ataata
```

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```
Stdxrcds  478 ---------------cg-----ca--------------------ccgc---
Padxrd    508 ---------------gg-----cc--------------------tgga---
Zmdxrd    482 ---------------cg-----catcataa-------------ccgc---
Sgdxrd    478 ---------------gg-----cg--------------------gcgc---
Nmdxrd    496 ---------------ta-----ca--------------------cagg---
Ecdxrd    482 ---------------cg-----ca--------------------acct---
Sldxrd    484 ---------------gggctttca--------------------acccatg
Mldxrd    490 ---------------gg-----ta--------------------cc-----
Pmdxrp    509 ---------------cg-----caaagacaaattgggttttgcccgc---
Atdxrd    718 ---------------ca-----ag--------------------gttt---
Cjdxrd    427 ---------------gg-----ta--------------------aaaa---
Pfdxrd    731 ataaggtattaaaaaca-----aa--------------------atgt---

Stdxrcds  486 --------------gcccagg--------ggcg---tccgccg-----ga
Padxrd    516 --------------gcgggtc--------ggcg---tgcgccg-----ga
Zmdxrd    496 --------------gacta------------tg---ttcgccg-----ga
Sgdxrd    486 --------------ccgcgcg--------gagg---tccgcaa-----gc
Nmdxrd    504 --------------tcgcctg--------aacg---aacacgg-----ca
Ecdxrd    490 --------------atccagcataatct-ggga---tacgctgaccttga
Sldxrd    500 ctgattttcggcctgctcaagtcgtggcagggc---tgcgacg-----ga
Mldxrd    496 ---------------cccgac--------gaag---ttgctaa-----gt
Pmdxrp    536 --------------tttctgaatta----ggga---tcagtaa-----ga
Atdxrd    726 --------------gcctgaa--------ggcgctctgcgcaa-----ga
Cjdxrd    435 --------------aaatata--------gcaa---aacttta-----ta
Pfdxrd    754 --------------ttacaag--------acaa---tttttct-----aa

Stdxrcds  506 tc------------------a--------------------tccttacc
Padxrd    536 tc------------------c--------------------tcttgacc
Zmdxrd    512 tt------------------a--------------------ttattacg
Sgdxrd    506 tg------------------g--------------------tggtgacc
Nmdxrd    524 tcgcttcgatt----------a--------------------tcctgacc
Ecdxrd    522 gc------------------aaaatggcgtggtgtccattttacttacc
Sldxrd    542 tt------------------c--------------------tcctgact
Mldxrd    515 ta------------------g--------------------tgctaacc
Pmdxrp    560 tt------------------g--------------------tgttaacg
Atdxrd    749 ta------------------a--------------------tcttgact
Cjdxrd    455 tc-----------------------------------------------aca
Pfdxrd    774 aattaacaatataaataaaata--------------------tttttatg
```

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```
Stdxrcds  517 gccagc-ggtggtccgttccgcg--cg----acgccgaaggaagcgatgc
Padxrd    547 gcctcc-ggcggcccgttccgcg--ag----acgccgctgga-gcaactc
Zmdxrd    523 gccagc-ggaggtcccttcagaa--ca----acgtctcttgccgaaatg-
Sgdxrd    517 gccagc-ggcggcccgttccgcaaccg----cacccgtgagcagc--tgg
Nmdxrd    544 gcttcc-ggcggcccgtttctga--c------cgccgatttaaac-acgt
Ecdxrd    553 gggtct-ggtggccctttccgtg--ag----acgcc--attgcgcgattt
Sldxrd    553 gccagt-ggcggcgcttttcggg--ac----tggccggtcgaacggctgt
Mldxrd    526 gcctcc-ggcgggccgtttcgtg--gctggaacgccg-gcgacttggagc
Pmdxrp    571 ggatcc-ggtggtccattccgtt--at----acccctctgga-gcaattt
Atdxrd    760 gcatct-ggtggagcttttaggg--at----tggcctgtcgaaaagctaa
Cjdxrd    460 gcaagt-ggtggagctttttata--gg----tataaaatcaaagatttaa
Pfdxrd    804 ttcatctggaggtccatttcaaa--at----ttaactatggacgaattaa

Stdxrcds  560 gcg-ac--a-tca---cccccgcacaggcggtggcg-catcccaactggt
Padxrd    589 gct-tc--ggtga---cgccggagcaggcttgtgcg-cacccgaactggt
Zmdxrd    565 gca-ac--ggtca---cgccagaacgcgcggttcag-catcccaactggt
Sgdxrd    560 cgg-cc--g-tca---cgccggccgacgcgctggcg-cacccgacctggg
Nmdxrd    584 tcg-ac--a-gcattacgcccgaccaagcggtcaaa-caccccaattggc
Ecdxrd    594 ggc-aacaa-tga---cgccggatcaagc-ctgccgtcatccgaactggt
Sldxrd    596 cgc-aa--g-taa---ctgtcgcagatgcgctcaag-catcccaactggt
Mldxrd    572 gcg-------tta---cacccgagcaggcgggcgtc-catccgacttggt
Pmdxrp    613 gaacag--a-tca---ccccagcacaagcagttgcg-catcctaattggt
Atdxrd    803 agg-aa--g-tta---aagtagcggatgcgttgaag-catccaaactgga
Cjdxrd    503 atc-aa--g-tca---gtgtcaaagatgctttaaaa-catcctaattgga
Pfdxrd    848 aaa-at--g-taa---catcagaaaatgctttaaag-catcctaaatgga

Stdxrcds  602 cgatgggcgccaagatctcggtcgactccgcgacgatgatgaacaagggg
Padxrd    632 cgatggggcgtaagatttccgtcgactccgccagcatgatgaacaagggg
Zmdxrd    608 caatgggtgccaagatttctatcgattctgctacaatgatgaataagggg
Sgdxrd    602 cgatgggcccggtggtgacgatcaactcggcgaccctggtgaacaagggc
Nmdxrd    629 gtatgggacgcaaaatctccgtcgattccgccaccatgatgaacaaaggt
Ecdxrd    638 cgatggggcgtaaaatttctgtcgattcggctaccatgatgaacaaaggt
Sldxrd    638 cgatggggcgcaagattaccgtcgactccgccaccttgatgaataaaggc
Mldxrd    611 caatggggacgatgaacacgctgaactcagcgtctctggttaacaagggg
Pmdxrp    656 caatggggaaaaagatctctgtcgattccgctaccatgatgaataaaggg
Atdxrd    845 acatgggaaagaaaatcactgtggactctgctacgcttttcaacaagggt
Cjdxrd    545 acatgggagcaaagatcactatagatagtgcgactatggcaaataagctt
Pfdxrd    890 aaatgggtaagaaaataactatagattctgcaactatgatgaataaaggt
```

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```
Stdxrcds  652 ctcgaactgatcgaagccttccacctgttcccggtcgcc--gccgagcaa
Padxrd    682 ctcgaactgatcgaggcgtgctggctgttc---gacgcccagccgagcca
Zmdxrd    658 cttgaattgatagaagcctatcatctcttccagattcca--ttagaaaaa
Sgdxrd    652 ctggaggtgatcgaggcgcacctgctgtacgacgtgccg--ttcgaccgg
Nmdxrd    679 ttggagctgattgaagcgcattggctgttcaactgtccg--cccgacaaa
Ecdxrd    688 ctggaatacattgaagcgcgttggctgtttaacgccagc--gccagccag
Sldxrd    688 ctcgaggtgatcgaagcccactatctcttcggcttggat--tacgactac
Mldxrd    661 ctcgagctcatcgaagccaacctgttgttcggcattccc--tacgaccgc
Pmdxrp    706 ttggaatatattgaagcacgctggttatttaatgcctcg--gcagaagaa
Atdxrd    895 cttgaggtcattgaagcgcattatttgtttggagctgag--tatgacgat
Cjdxrd    595 tttgagattatagaggcttatcatttat------atgat--tttaaagaa
Pfdxrd    940 ttagaggttatagaaacccatttttttatttgatgtagat--tataatgat

Stdxrcds  700 c-tggccgtgctggtccatcgccaatccgtcgtccattcgatggtggaat
Padxrd    729 ggtcgaggtggtgatccacccgcagagcgtgatccactcgatggtggact
Zmdxrd    706 t-ttgaaattttggttcatcctcagtcagttattcactccatggtggaat
Sgdxrd    700 a-tcgaggtggtggtccatccgcagtcggtcgttcattcgatggtggaat
Nmdxrd    727 c-tcgaagtcgtcatccatccgcaatctgtgatacacagcatggtgcgct
Ecdxrd    736 a-tggaagtgctgattcacccgcagtcagtgattcactcaatggtgcgct
Sldxrd    736 a-tcgacatcgtcatccatccccagagcatcatccactcgctgattgagc
Mldxrd    709 a-ttgaggtggttgtgcaccctcagtcaattgttcattcgatggtgacat
Pmdxrp    754 a-tggaagttattattcatcctcaatccattattcattctatggtacgtt
Atdxrd    943 a-tagagattgtcattcatccgcaaagtatcatacattccatgattgaaa
Cjdxrd    637 a-ttgatgctttaatagaaccaagatctttagtgcatgcaatgtgtgaat
Pfdxrd    988 a-tagaagttatagtacataaagaatgcattatacattcttgtgttgaat

Stdxrcds  749 atgtcgacggatcggtgctggcccagctcggcacgcccgacatgcgcacg
Padxrd    779 acgtcgacggttcggtgatcgcccagctcggcaatccggacatgcgcacg
Zmdxrd    755 atttggatggttctatccttgcccagatcggtagtcctgatatgagaaca
Sgdxrd    749 tcgtggacggttcgacgatggcccaggccagcccgccggacatgcgcatg
Nmdxrd    776 accgcgacggctccgtgttggcgcaactgggcaatcccgatatgcgaacg
Ecdxrd    785 atcaggacggcagtgttctggcgcagctgggggaaccggatatgcgtacg
Sldxrd    785 tagaagatacctccgtcttggcgcaattgggctggccggatatgcgactg
Mldxrd    758 tcatcgacggctcgacgatcgcccaagccagccctccggacatgaagcta
Pmdxrp    803 acatcgatgggtccgtgattgctcaaatggggaatcctgatatgcgtaca
Atdxrd    992 cacaggattcatctgtgcttgctcaattgggttggcctgatatgcgttta
Cjdxrd    686 ttaaaaatggagctagcacggcgtatttttcaaaagcagatatgaaacta
Pfdxrd   1037 ttatagacaaatcagtaataagtcaaatgtattatccagatatgcaaata
```

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```
Stdxrcds  799 ccgatcgcctatgcgctggcttggcccgagcgga----t----------g
Padxrd    829 ccgatttcctatgccatggcctggccggagcgaa----t----------c
Zmdxrd    805 ccgatcggtcatactttggcttggccaaagcgga----t----------g
Sgdxrd    799 ccgatcgcgctgggcctcggctggccggaccggg----t----------g
Nmdxrd    826 cctatcgcttattgtttgggtttgcccgagcgca----t----------c
Ecdxrd    835 ccaattgcccacaccatggcatggccgaatcgcg----t----------g
Sldxrd    835 cccttgctctacgccctctcctggcccgatcgcc----t----------c
Mldxrd    808 cctatttctttggcgttgggctggccacagcggg----t----------g
Pmdxrp    853 ccgattgcggaaaccatggcatatccaagtcggaccgtt----------g
Atdxrd   1042 ccgattctctacaccatgtcatggcccgatagag----ttccttgttctg
Cjdxrd    736 gctatttcagatgctatattt------gaaaaac----a----------a
Pfdxrd   1087 cccatattatattctttaacatggcctgatagaa----t----------a

Stdxrcds  835 gag-acgc---tgtgccc----gccgc-t-cgaccttg--------ccac
Padxrd    865 gat-tccg---gcgtttc----gccgc-t-ggatatgt--------tcgc
Zmdxrd    841 gaa-acac---cagccga----atcgt-t-ggatttta--------ccaa
Sgdxrd    835 ccggacgc-----cgccc----ccggc-tgcgactgga--------ccaa
Nmdxrd    862 gat-tcgg----gtgtcg----gcgacct-ggatttcg--------acgc
Ecdxrd    871 aa-----c---tctggcgtgaagccgc-t-cgattttt--------gcaa
Sldxrd    871 tct-actc---aatggtc----ggcgc-t-cgatctgg--------tcaa
Mldxrd    844 g----------gtg-gc----gctgc-t-cgagcctgtgctttcactac
Pmdxrp    893 ctg-gcgt---tgagccc--------t-t-ggattttt--------acca
Atdxrd   1088 aag-taac---t-tggcc----aagac-t-tgaccttt--------gcaa
Cjdxrd    766 gat-acgcctattttaga----ggctg-t-tgatttta--------gca-
Pfdxrd   1123 aaa-acaa---atttaaa----acctt-t-agatttgg--------ctca

Stdxrcds  867 ggtgggtaagctcgagttcgaaaatcccgatctcgatcgcttc-------
Padxrd    897 cgtcggtcgcctggatttccagcgccccgacgagcagcgcttc-------
Zmdxrd    873 attgcgccagatggattttgaagcaccagattatgaacgtttt-------
Sgdxrd    867 ggccgcgacctgggagttcttcccgctggacaacgaggcgttc-------
Nmdxrd    894 attgtccgcgctgaccttccaaaagcccgactttgaccgcttc-------
Ecdxrd    903 actaagtgcgttgacatttgccgcaccggattatgatcgttat-------
Sldxrd    903 agcgggcagcttggagttccgggaaccggatcacgccaaatac-------
Mldxrd    876 cgcatctacctgggaattcgagccgctggacatcgatgttttt-------
Pmdxrp    921 actgaatggattaacctttattgagccagactatcaacgttat-------
Atdxrd   1119 actcggttcattgactttcaagaaaccagacaatgtgaaatac-------
Cjdxrd    800 -------aaatgcctgctttaaaatttc-atc-caatcagcacaaaaaaa
Pfdxrd   1155 ggtttcaactcttacatttcataaaccttctttagaacatttc-------
```

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```
Stdxrcds  910 ---ccggcgctcgcgctggcgatggaggcattgaag-gcgggcggggcgc
Padxrd    940 ---ccctgcctgcgcctggcgagccaggccgcggaa-accggcggcagcg
Zmdxrd    916 ---ccggcattaactttggcaatggaatccatcaaa-tcaggtggggctc
Sgdxrd    910 ---ccggcggtcgagctggcccgcgaggtgggtacg-ctcggcgggaccg
Nmdxrd    937 ---ccctgcctgaagctcgcctatgaagccatgaac-gcaggcggagccg
Ecdxrd    946 ---ccatgcctgaaactggcgatggaggcgttcgaa-caaggccaggcag
Sldxrd    946 ---ccctgcatggacttggcctacgccgccggtcgc-aaaggcggcacaa
Mldxrd    919 ---cccgcagtcgagctggcccggcacgctggacag-atcggcggctgta
Pmdxrp    964 ---ccttgtttaaaattagctattgacgcattttca-gccggacaatatg
Atdxrd   1162 ---ccatccatggatcttgcttatgctgc-tggacgagctggaggcacaa
Cjdxrd    841 tatcctatttttaagcttaaaaatacatttttaaaa-gagccaaatttag
Pfdxrd   1198 ---ccgtgtattaaattagcttatcaagcaggtata-aaaggaaacttttt

Stdxrcds  956 gtccggccattctcaatgccgccaacgaagtcgccgtcgcggcctttctc
Padxrd    986 ccccggccatgctgaatgccgcgaacgaggtggccgtggccgcatttctc
Zmdxrd    962 gtcctgctgtaatgaatgccgctaatgaaatagctgtggcggccttcctt
Sgdxrd    956 ccccggcggtcttcaatgccgccaacgaggaatgtgtggacg-ctttcct
Nmdxrd    983 cgccctgcgtattgaacgccgccaacgaagccgccgtcgccgccttttttg
Ecdxrd    992 cgacgacagcattgaatgccgcaaacgaaatcaccgttgctgcttttctt
Sldxrd    992 tgccagccgtcttgaatgcggcgaatgagcaagccgtcgccctcttccta
Mldxrd    965 tgaccgccatttacgatgctgctaatgaggaggctgcagaggccttcctc
Pmdxrp   1010 ccacgacagcaatgaatgcagcgaatgaaatcgcggtagcgtctttctta
Atdxrd   1208 tgactggagttctcagcgccgccaatgagaaagctgttgaaatgttcatt
Cjdxrd    890 gt---gttatcatcaatgctgctaatgaagttggtgtttataatttttta
Pfdxrd   1244 atccaactgtactaaatgcgtcaaatgaaatagctaacaacttatttttg

Stdxrcds 1006 gccgggcggat----------c--------ggattccttgaaa-ttgccg
Padxrd   1036 gagcggcacat----------c--------cgcttcagcgaca-tcgcgg
Zmdxrd   1012 gataagaaaat----------c--------ggttttcttgata-tcgcta
Sgdxrd   1005 gaagggcgcactgcccttcacc--------ggaatcgtggaca-ctgtgg
Nmdxrd   1033 gacggacagat----------t--------aagtttaccgaca-ttgcca
Ecdxrd   1042 gcgcaacaaat----------c--------cgctttacggata-tcgctg
Sldxrd   1042 gaggagcaaat----------t--------cacttctcggata-ttccgc
Mldxrd   1015 caaggtcggat----------c--------ggcttccccgcca-tcgtcg
Pmdxrp   1060 gacaataagat----------t--------aaattcacagata-ttg---
Atdxrd   1258 gatgaaaagat----------aagctatttggatatcttcaaggttgtgg
Cjdxrd    937 gaaaataaaag----------t--------ggatttttagaca-ttgcta
Pfdxrd   1294 aataataaaat----------t--------aaatattttgata-tttcct
```

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```
Stdxrcds 1037 c----aatctctgccg----atacgctgtctcgctatgac----ccgg--
Padxrd   1067 t----tatcatcgagg----acgtgctgaaccgcgaggcg----gtga--
Zmdxrd   1043 a----aattgtcgaga----aaacattagatcattataca----cccg--
Sgdxrd   1046 c----gaaggtggtcgccgaacacggcacaccgcaat--------cgg--
Nmdxrd   1064 a----aaccgtcgccc----attgtctttcac---aagacttttcaga--
Ecdxrd   1073 cgttgaatttatccgt----a----ctggaaaaaatggat----atgc--
Sldxrd   1073 g----cctgattgaac----gtgcctgcgatcgccaccaa----acgg--
Mldxrd   1046 c----aacaatcgcgg----atgtgttgcagcgtgccgac----caat--
Pmdxrp   1088 --------cgcgacta----aatcagttagtcgtgagcaa----attg--
Atdxrd   1298 a----attaacatgcg----ataaac---atcgaaacgag----ttggta
Cjdxrd    968 a----atgcattttta----aagcccttgatcattttgga----gtac--
Pfdxrd   1325 ctat-aatatcgcaag----ttcttgaatctttcaattct----caaa--

Stdxrcds 1073 -ccgcgcc---g------gaaacgc-------tc---g------atg---
Padxrd   1103 -ccgcagt---c------gaatcgc-------tc---g------atc---
Zmdxrd   1079 -caacccc---g------tcttctt-------tg---g------aag---
Sgdxrd   1082 -gaacttc---g------ctcacgg-------tg---g------agg---
Nmdxrd   1101 -cggcata---g------gcgac-a-------ta---g------ggg---
Ecdxrd   1109 -gcgaacc---a------caatgtg-------tg---g------acg---
Sldxrd   1109 -agtggcaacag------caaccga-------gcttgg------atg---
Mldxrd   1082 -gggctcc---c------caatggg-------gt---g------agggac
Pmdxrp   1120 -caaccac---a------aaaaattcattgcata---g------aag---
Atdxrd   1333 acatcacc---gtctcttgaagaga-------tt---gttcactatg---
Cjdxrd   1004 -ctaaaat---t------tcaagca-------ta---g------aag---
Pfdxrd   1364 -aggtttc---g------gaaaata-------gt---g------aag---

Stdxrcds 1094 ccg----tgctggc----g------------atcga------cgc--gga
Padxrd   1124 agg----tcctggctgccg------------atcgc------cgc-----
Zmdxrd   1100 atg----tctttgc----g------------atcga------caa--tga
Sgdxrd   1103 acg----tac----------------------tcca------cgc--gga
Nmdxrd   1121 ggc----tcttggc----g------------caaga------tgcccgga
Ecdxrd   1130 atg----tgttatc----t------------gttga------tgc--gaa
Sldxrd   1136 aca----ttttggc----c------------tacga------cgc--ttg
Mldxrd   1106 ccgctactgtggat----g------------atgta------ctc---ga
Pmdxrp   1148 atg----tacttga----g------------gtaga------taa--aaa
Atdxrd   1367 act----tgtgggc----a------------cgtgaatatgccgc--gaa
Cjdxrd   1025 aag----tttttga----g------------tatga--------------
Pfdxrd   1385 att----taatgaa----gcaaattctacaaataca------ttc--ttg
```

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```
Stdxrcds 1116 g-gcgcggc--tttacgcggctgagcg-agtg------------------
Padxrd   1147 --gcgcg-----ttcggtcgccgggca-atgg------------------
Zmdxrd   1122 a-gcgcgga--tacaagccgctgcttt-aatg------------------
Sgdxrd   1119 gagctgggc-----ccgggcccgggcc-cgcg------------------
Nmdxrd   1145 c-acgcgca--caagcgcgg----gca-ttta------------------
Ecdxrd   1152 c-gcgcg----tgaagtcgccaga----aaag------------------
Sldxrd   1158 g-gcacggcagtttgtgcaagctagct-atca------------------
Mldxrd   1131 c-gcgcagc--gctgggcccgtgagcg-agcgttgtgtgcggtagcaaca
Pmdxrp   1170 g-gcaagggaattatctcagtcaatca-tttt------------------
Atdxrd   1395 t-gtgcagc--tttcttctg--gtgct-aggc------------------
Cjdxrd   1041 -----------ttttaaaacaagagagtattt------------------
Pfdxrd   1419 g-gccaaag--ataaagctaccgatat-atac------------------

Stdxrcds 1144 ------------aag----gactgc--gtcg----cttga----------
Padxrd   1171 ------------ttg----acccgg--cacg----ccggctag-------
Zmdxrd   1150 ------------gag----agtttg--cccg----cgtga----------
Sgdxrd   1145 ------------a-------gctggcggccg----gctga----------
Nmdxrd   1169 ------------tcg----gcacac--tgcg----c-tga----------
Ecdxrd   1175 ------------agg----tgatgc--gtct----cgcaagctga-----
Sldxrd   1188 ------------aagtctggaatcc--gtcg----tttag----------
Mldxrd   1177 gcgagttctggaaag----gtctct--gacatggtcttagaaaggtccta
Pmdxrp   1200 ------------aag----tttttc--acat----ccgtaa---------
Atdxrd   1421 ------------cag-------ttc--at-g----catga----------
Cjdxrd   1062 ------------aag----ga--------------gttaa----------
Pfdxrd   1447 ------------aac----aaacat--aatt----cttcatag-------

Stdxrcds 1162 -
Padxrd   1192 -
Zmdxrd   1168 -
Sgdxrd   1162 -
Nmdxrd   1186 -
Ecdxrd   1198 -
Sldxrd   1210 -
Mldxrd   1221 a
Pmdxrp   1219 -
Atdxrd   1435 -
Cjdxrd   1072 -
Pfdxrd   1468 -
```

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```
Stdxrp      1 --------------------------------------------------
Zmdxrp      1 ms------------------------------------------------
Padxrp      1 ms------------------------------------------------
Ecdxrp      1 --------------------------------------------------
Nmdxrp      1 m-------------------------------------------------
Hidxrp      1 m-------------------------------------------------
Ssdxrp      1 --------------------------------------------------
Pmdxrp      1 msisy----------------------------------------------
Sldxrp      1 --------------------------------------------------
Sgdxrp      1 --------------------------------------------------
Bsdxrp      1 --------------------------------------------------
Mldxrp      1 mnn------------------------------------------------
Mtdxrp      1 matggrv--------------------------------------------
Atdxrp      1 mmtlnslspaeskaisfldtsrfnpipklsggfslrrrnqgrgfgkgvkc
Cjdxrp      1 --------------------------------------------------
Pfdxrp      1 mkkyiyiyfffititindlvinntskcvsierrknnayinygigyngpdn

Stdxrp      1 ----------------------------vvk-------r-----------
Zmdxrp      3 ----------------------------qpr-------t-----------
Padxrp      3 ----------------------------rpq-------r-----------
Ecdxrp      1 ----------------------------mk-------q-----------
Nmdxrp      2 ----------------------------tpq-------v-----------
Hidxrp      2 ----------------------------qkq-------n-----------
Ssdxrp      1 ----------------------------mvk-------r-----------
Pmdxrp      6 ----------------------------fmk-------k-----------
Sldxrp      1 ----------------------------mk-------a-----------
Sgdxrp      1 --------------------------------------------------
Bsdxrp      1 ----------------------------mk-------n-----------
Mldxrp      4 ----------------------------pieghaggrlr----------
Mtdxrp      8 ----------------------------vir-------rrgdnevvahnd
Atdxrp     51 svkvqqqqqpppawpgravpeaprqswdgpk-------p-----------
Cjdxrp      1 --------------------------------------------------
Pfdxrp     51 kitksrrckriklckkdlidig------aik-------kpin--------
```

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```
Stdxrp      5 ---------------vtvlgatgsvgtstldlie---rnphafevvalta
Zmdxrp      7 ---------------vtvlgatgsighstldlie---rnldryqvialta
Padxrp      7 ---------------isvlgatgsiglstldvvq---rhpdryeafaltg
Ecdxrp      4 ---------------ltilgstgsigcstldvvr---hnpehfrvvalva
Nmdxrp      6 ---------------ltilgstgsigestldvvs---rhpekfrvfalag
Hidxrp      6 ---------------ivilgstgsigkstlsvie---nnpqkyhafalvg
Ssdxrp      5 ---------------isilgstgsigtqtldivt---hhpdafqvvglaa
Pmdxrp     10 ---------------ivilgstgsigtstlsvit---hnpdkyqvfalvg
Sldxrp      4 ---------------vtllgstgsigtqtldile---qypdrfrlvglaa
Sgdxrp      1 ---------------mvilgstgsigtqaidvvl---rnpgrfkvvalsa
Bsdxrp      4 ---------------icllgatgsigeqtldvlr---ahqdqfqlvsmsf
Mldxrp     15 ---------------vlvlgstgsigtqalevia---anpdrfevvglaa
Mtdxrp     23 evtnstdgradgrlrvvvlgstgsigtqalqvia---dnpdrfevvglaa
Atdxrp     83 ---------------isivgstgsigtqtldiva---enpdkfrvvalaa
Cjdxrp      1 ---------------milfgstgsigvnalklaa---lk--nipisalac
Pfdxrp     80 ---------------vaifgstgsigtnalniirecnkienvfnvkalyv

Stdxrp     37 -n-cdveklaaaairtrarcavvadekclpalqerla--g----s----g
Zmdxrp     39 -n-rnvkdladaakrtnakraviadpslyndlkeala--g----s----s
Padxrp     39 -f-srlaelealclrhrpvyavvpeqaaaialqggsla--a----a----g
Ecdxrp     36 -g-knvtrmveqclefspryavmddeasakllktmlqqqg----s----r
Nmdxrp     38 -h-kqveklaaqcqtfhpeyavvadaehaarleallkrdg----t----a
Hidxrp     38 -g-knveamfeqcikfrphfaalddvnaakilrekli--a----h----h
Ssdxrp     37 -g-gnvallaqqvaefrpeivairqaekledlkaava--el---t----d
Pmdxrp     42 -g-rnvelmfqqcltfqpsfaaldddvaakmlaeklk--ahq--s----q
Sldxrp     36 -g-rnvallseqirrhrpeivaiqdaaqlselqaaia--dld--n----p
Sgdxrp     33 ag-gavellaeqavalgvhtvavad----paaeeaaar-g----p----g
Bsdxrp     36 -g-rnidkavpmievfqpkfvsvgdldtyhklkqmsf--s----f----e
Mldxrp     47 -ggaqldtllrqraatgvtniaiaddra-----aqla--g----dipyhg
Mtdxrp     70 -ggahldtllrqraqtgvtniavadehaaqrvgd---------------
Atdxrp    115 -g-snvtlladqvrrfkpalvavrneslinelkeala--d----l----d
Cjdxrp     31 -g-dniallneqiarfkpkfvsikdsknkhlvkhdrv--f----i----g
Pfdxrp    115 -n-ksvnelyeqareflpeylcihdksvyeelkelvk--nikdyk----p
```

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```
Stdxrp    75 --v--ea--mg--------gahsvcdva-rm-g-adwtmaa-ivgsaglk
Zmdxrp    77 --v--ea--aa--------gadalveaa-mm-g-adwtmaa-iigcaglk
Padxrp    77 --i--rtrvlf--------geqalceva-sa-pevdmvmaa-ivgaaglp
Ecdxrp    76 --t--ev--ls--------gqqaacdma-aled-vdqvmaa-ivgaagll
Nmdxrp    78 --t--qv--lh--------gaqalvdva-sa-devsgvmca-ivgavglp
Hidxrp    76 --iptev--la--------grraicelaahp-d-adqimas-ivgaagll
Ssdxrp    76 --y--qp--myvv------geegvveva-ry-gdaesvvtg-ivgcagll
Pmdxrp    82 --t--tv--la--------gqqaicelaahp-e-admvmaa-ivgaagll
Sldxrp    76 --p--li--lt--------geagvteva-ry-gdaeivvtg-ivgcagll
Sgdxrp    69 --g--qg--agrplprvlagpdaatela-aa-e-chsvlng-itgsigla
Bsdxrp    74 --c--qi--gl--------geeglieaa-vm-eevdivvna-llgsvgli
Mldxrp    85 --t--da--vt--------rl-----ve-et-e-advvlna-lvgalglr
Mtdxrp   103 --i--py--hg--------sdaatrlve-qt-e-advvlna-lvgalglr
Atdxrp   153 ykl--ei--ip--------geqgvieva-rh-p-eavtvvtgivgcaglk
Cjdxrp    69 --q--eg--le--------qiltecqdk-ll-------lna-ivgfaglk
Pfdxrp   157 --i--il--cgde------gmkeic--s-sn-s-idkivig-idsfqgly

Stdxrp   107 pvmaaleaggtvalankeslvsagevmmaaarah-gatllpvdsehnavf
Zmdxrp   109 atlaairkgktvalankeslvsagglmidavreh-gttllpvdsehnaif
Padxrp   112 stlaaveagkrvllankealvmsgalfmqavkrs-gavllpidsehnaif
Ecdxrp   109 ptlaairagktillankeslvtcgrlfmdavkqs-kaqllpvdsehnaif
Nmdxrp   111 salaaaqkgktiylanketlvvsgalfmetaran-gaavlpvdsehnavf
Hidxrp   111 ptlsavkagkrvllankeslvtcgqlfidavkny-gskllpvdsehnaif
Ssdxrp   111 ptmaaiaagkdialanketliagapvvlplvekm-gvkllpadsehsaif
Pmdxrp   115 ptlsavkagkrvllankealvtcgqlfidavres-qaqllpvdsehnaif
Sldxrp   109 ptiaaieagkdialanketliaagpvvlpllqkh-gvtitpadsehsaif
Sgdxrp   109 ptlaalragrvlvlankeslivggplvkavaqp---gqivpvdsehaalf
Bsdxrp   107 ptlkaieqkktialanketlvtaghivkehakky-dvpllpvdsehsaif
Mldxrp   112 ptlaalhtgarlalankeslvaggslvlaaaqp---gqivpvdsehsala
Mtdxrp   135 ptlaalktgarlalankeslvaggslvlraarp---gqivpvdsehsala
Atdxrp   188 ptvaaieagkdialanketliaggpfvlplankh-nvkilpadsehsaif
Cjdxrp    96 stlkakelgknialankeslvvagsfl------k-gakflpvdsehaalk
Pfdxrp   189 stmyaimnnkivalankesivsagfflkkllnihknakiipvdsehsaif
```

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```
Stdxrp   156 qcldrtap----------r----------g-----vrriiltasggp
Zmdxrp   158 qcfphhnr----------d----------y-----vrriiitasggp
Padxrp   161 qslprnya----------d----------glervgvrrilltasggp
Ecdxrp   158 qslpqpiq----------hnlgyadleqng-----vvsilltgsggp
Nmdxrp   160 qvlprdytgrlne--------h----------g-----iasiiltasggp
Hidxrp   160 qslppeaq----------ekigfcplselg-----vskiiltgsggp
Ssdxrp   160 qclqgvpe----------g----------g-----lrriiltasgga
Pmdxrp   164 qslppeaq----------rqigfcplselg-----iskivltgsggp
Sldxrp   158 qciqglst----------hadfrpaqvvag-----lrrilltasgga
Sgdxrp   156 qalaggar----------a----------e-----vrklvvtasggp
Bsdxrp   156 qalqgeqa----------k----------n-----ierliitasggs
Mldxrp   159 qclrggtp----------d----------e-----vaklvltasggp
Mtdxrp   182 qclrggtp----------d----------e-----vaklvltasggp
Atdxrp   237 qciqglpe----------g----------a-----lrkiiltasgga
Cjdxrp   139 flle--gk----------k----------n-----iaklyitasgga
Pfdxrp   239 qcldnnkvlktkclqdnfskin----------n-----inkiflcssggp

Stdxrp   178 fratpkeamrditpaqavahpnwsmgakisvdsatmmnkglelieafhlf
Zmdxrp   180 frttslaematvtperavqhpnwsmgakisidsatmmnkglelieayhlf
Padxrp   188 fretpleqlasvtpeqacahpnwsmgrkisvdsasmmnkglelieacwlf
Ecdxrp   190 fretplrdlatmtpdqacrhpnwsmgrkisvdsatmmnkgleyiearwlf
Nmdxrp   187 fltadlntfdsitpdqavkhpnwrmgrkisvdsatmmnkglelieahwlf
Hidxrp   192 frytpleqftnitpeqavahpnwsmgkkisvdsatmmnkgleyiearwlf
Ssdxrp   182 frdlpverlpfvtvqdalkhpnwsmgqkitidsatlmnkglevieahylf
Pmdxrp   196 frytpleqfeqitpaqavahpnwsmgkkisvdsatmmnkgleyiearwlf
Sldxrp   190 frdwpverlsqvtvadalkhpnwsmgrkitvdsatlmnkglevieahylf
Sgdxrp   178 frnrtreqlaavtpadalahptwamgpvvtinsatlvnkglevieahlly
Bsdxrp   178 frdktreelesvtvedalkhpnwsmgakitidsatmmnkglevieahwlf
Mldxrp   181 frgwnagdlervtpeqagvhptwsmgtmntlnsaslvnkglelieanllf
Mtdxrp   204 frgwsaadlehvtpeqagahptwsmgpmntlnsaslvnkgleviethllf
Atdxrp   259 frdwpveklkevkvadalkhpnwnmgkkitvdsatlfnkglevieahylf
Cjdxrp   159 fyrykikdlnqvsvkdalkhpnwnmgakitidsatmanklfeiieayhly
Pfdxrp   274 fqnltmdelknvtsenalkhpkwkmgkkitidsatmmnkgleviethflf
```

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```
Stdxrp   228 pvaaeqlavlvhrqsvvhsmveyvdgsvlaqlgtpdmrtpiayalawper
Zmdxrp   230 qiplekfeilvhpqsvihsmveyldgsilaqigspdmrtpightlawpkr
Padxrp   238 daqpsqvevvihpqsvihsmvdyvdgsviaqlgnpdmrtpisyamawper
Ecdxrp   240 nasasqmevlihpqsvihsmvryqdgsvlaqlgepdmrtpiahtmawpnr
Nmdxrp   237 ncppdklevvihpqsvihsmvryrdgsvlaqlgnpdmrtpiayclglper
Hidxrp   242 nasaeemeviihpqsiihsmvryvdgsvitqmgnpdmrtpiaetmayphr
Ssdxrp   232 gldydhidivihpqsiihslievqdtsvlaqlgwpdmrlpllyalswper
Pmdxrp   246 nasaeemeviihpqsiihsmvryidgsviaqmgnpdmrtpiaetmaypsr
Sldxrp   240 gldydyidivihpqsiihslieledtsvlaqlgwpdmrlpllyalswpdr
Sgdxrp   228 dvpfdrievvvhpqsvvhsmvefvdgstmaqasppdmrmpialglgwpdr
Bsdxrp   228 dipyeqidvvlhkesiihsmvefhdksviaqlgtpdmrvpiqyaltypdr
Mldxrp   231 gipydrievvvhpqsivhsmvtfidgstiaqasppdmklpislalgwpqr
Mtdxrp   254 gipydridvvvhpqsiihsmvtfidgstiaqasppdmklpislalgwprr
Atdxrp   309 gaeyddieivihpqsiihsmietqdssvlaqlgwpdmrlpilytmswpdr
Cjdxrp   209 df--keidalieprslvhamcefkngastayfskadmklaisdaif--ek
Pfdxrp   324 dvdyndievivhkeciihscvefidksvisqmyypdmqipilysltwpdr

Stdxrp   278 m---et-l-cppldlatvgklefenpdldrfpalalamealkaggarpai
Zmdxrp   280 m---et-p-aesldftklrqmdfeapdyerfpaltlamesiksggarpav
Padxrp   288 i---ds-g-vspldmfavgrldfqrpdeqrfpclrlasqaaetggsapam
Ecdxrp   290 v---ns-g-vkpldfcklsaltfaapdydrypclklameafeqgqaatta
Nmdxrp   287 i---ds-g-vgdldfdalsaltfqkpdfdrfpclklayeamnaggaapcv
Hidxrp   292 t---fa-g-vepldffkikeltfiepdfnrypnlklaidafaagqyatta
Ssdxrp   282 i---yt-d-wepldlvkagslsfrepdhdkypcmqlaygagraggampav
Pmdxrp   296 t---va-g-vepldfyqlngltfiepdyqrypclklaidafsagqyatta
Sldxrp   290 l---st-q-wsaldlvkagslefrepdhakypcmdlayaagrkggtmpav
Sgdxrp   278 v---pd-a-apgcdwtkaatweffpldneafpavelarevgtlggtapav
Bsdxrp   278 l---pl-pdakrlelweigslhfekadfdrfrclqfafesgkiggtmptv
Mldxrp   281 v---gg-a-aracafttastwefepldidvfpavelarhagqiggcmtai
Mtdxrp   304 v---sg-a-aaacdfhtasswefepldtdvfpavelarqagvaggcmtav
Atdxrp   359 vpcsev-t-wprldlcklgsltfkkpdnvkypsmdlayaagraggtmtgv
Cjdxrp   255 q---dtpi-leavdfskmpalkfhpistkkypifklkntflkepnl-gvi
Pfdxrp   374 i---kt-n-lkpldlaqvstltfhkpslehfpciklayqagikgnfyptv
```

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```
Stdxrp   323 lnaanevavaaflagrigfleiaaisadtlsry---d---pa-a--pe--
Zmdxrp   325 mnaaneiavaafldkkigfldiakivektldhy---t---pa-t--ps--
Padxrp   333 lnaanevavaaflerhirfsdiaviiedvlnre---a---vt-a--ve--
Ecdxrp   335 lnaaneitvaaflaqqirftdiaalnlsvlekm---d---mr-e--pq--
Nmdxrp   332 lnaaneaavaafldgqikftdiaktvahclsqd---f---sd-g--ig--
Hidxrp   337 mnaaneiavqafldrqigfmdiakinsktieri---s---py-t--iq--
Ssdxrp   327 lnaaneqavalflqekisfldiprliektcdlyvgqn---ta-s--pd--
Pmdxrp   341 mnaaneiavasfldnkikftdiarlnqlvvskl---q---pq-k--ih--
Sldxrp   335 lnaaneqavalfleeqihfsdiprlieracdrh---q---te-w--qqqp
Sgdxrp   323 fnaaneecvdaflkgalpftgivdtvakvvaeh---gt--pq-s--gt--
Bsdxrp   324 lnaanevavaaflagkipflaiedciekaltrh---qllkkp-s--wr--
Mldxrp   326 ydaaneeaaeaflqgrigfpaivatiadvlqra---d---qw-a--pq--
Mtdxrp   349 ynaaneeaaaaflagrigfpaivgiiadvlhaa---d---qw-avepa--
Atdxrp   407 lsaanekavemfidekisyldifkvveltcdkhrn-e---lv-t--sp--
Cjdxrp   300 inaanevgvynflenksgfldiakcifkaldhf---g---vp-k--is--
Pfdxrp   419 lnasneiannlflnnkikyfdissiisqvlesf---n---sqkv--se--

Stdxrp   362 tldavlaid--aearlyaaervkdcva----------------------
Zmdxrp   364 sledvfaid--neariqaaalmeslpa----------------------
Padxrp   372 sldqvlaad--rrarsvagqwltrhag----------------------
Ecdxrp   374 cvddvlsvd--anarevarkevmrlas----------------------
Nmdxrp   371 digqllaqd--artraqarafigtlr----------------------
Hidxrp   376 niddvleid--aqareiaktllre----------------------
Ssdxrp   369 -letilaad--qwarrtvlen-sacvatrp----------------------
Pmdxrp   380 ciedvlevd--kkarelsqsiilsfshp----------------------
Sldxrp   376 slddilayd--awarqfvqasyqslesvv----------------------
Sgdxrp   363 sltvedvlh--aes--warararelaag----------------------
Bsdxrp   366 tfkkwtkip--gdtsiqyshkvv-cs----------------------
Mldxrp   365 wgegpatvddvldaqrwareralcavatassgkvsdmvlers--------
Mtdxrp   390 tvddvl------daqrwareraqravsgmasvaiastakpgaagrhastl
Atdxrp   448 sleeivhyd--lwareyaanvqlssgarpvha----------------
Cjdxrp   339 sieevfeyd--fktreylrs----------------------------
Pfdxrp   459 nsedlmkqi--lqihswakdkatdiynkhn----------------
```

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```
Stdxrp   387 ---
Zmdxrp   389 ---
Padxrp   397 ---
Ecdxrp   399 ---
Nmdxrp   395 ---
Hidxrp   398 ---
Ssdxrp   395 ---
Pmdxrp   406 ---
Sldxrp   403 ---
Sgdxrp   387 ---
Bsdxrp   389 ---
Mldxrp   407 ---
Mtdxrp   434 ers
Atdxrp   478 ---
Cjdxrp   357 ---
Pfdxrp   487 ---
```